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THE ROLE OF SLUDGE WORMS IN
EUTROPHICATION

Ralph O. Brinkhurst

National Environmental Research Center
Corvallis, Oregon

August 1972

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Ecological Research Series

The Role of Sludge Worms in Eutrophication



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THE ROLE OF SLUDGE WORMS IN EUTROPHICATION

By

Ralph O. Brinkhurst

Project 16010 ECQ

Project Officer

Charles F. Powers
Environmental Protection Agency
National Environmental Research Center
200 S.W. 35th Street
Corvallis, Oregon 97330

Prepared for

OFFICE OF RESEARCH AND MONITORING
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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ABSTRACT

In grossly polluted Toronto Harbour, Lake Ontario the worm population averaging 96,000 animals/m² (18.3 g ash-free dry wt/m²) assimilates 1,743 kcals/m²/yr and produces 1550 kcals/m²/yr worm tissue. Of the total nitrogen input of 830 tons/year, 7 tons is present in worm tissue at any one time and 113 tons may be circulated in a year. Production and Respiration values for tubificids should be based on mixed species cultures because of positive interactions between species which increase the assimilation rate and assimilation efficiency. Worms feed selectively upon the bacteria in sediment. They also pump water through sediment. Large worm populations play a significant role in preventing organic matter from being deposited in an energy or material sink. The quality or specific identity of organic matter inputs are of as much interest as their total calorific or carbon content in determining their effect on benthic production.

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16. Abstract In grossly polluted Toronto Harbour, Lake Ontario, worm populations ($\bar{x}$ 96,000/m ² , 18.3 g AF dry wt./m ²) assimilates 1,743 kcals/m ² /yr, produces 1550 kcals, 7 tons nitrogen standing stock of worms circulates 830 tons/yr of total input 830 tons. Production and respiration values for mixed species cultures differ markedly from those of pure cultures. Worms feed selectively, digest bacteria selectively, actively irrigate sediments. They retrieve materials from the energy sink via production and irrigation. Production of benthos depends on quality of sedimenting material, not just total energy or material content. (<i>EPA abstract</i>)				
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SECTION I

CONCLUSIONS

1. Production and assimilation estimates of benthic organisms made by reference to the Ivlev formula $\text{Ingestion} = \text{Production} + \text{Respiration} + \text{Egestion} + \text{Excretion}$ should be made on species mixtures as found in nature because of interspecific interactions.
2. The quality of sedimented material is important in determining material and energy sources available to the benthos via the microbiota as some material is relatively refractile while some is highly labile.
3. At densities as high as 18.3 g ash-free dry weight of worms/ m^2 (96,000 worms/ m^2) assimilation may reach 1,743 kcals/ m^2/yr production 1,550 kcals/ m^2/yr .
4. Assuming that the input of N_2 of 830 tons/annum is evenly mixed throughout Toronto Harbour, the standing stock of worms represents 7 tons, the throughput represents 113 tons. As the available nitrogen enters at one point in the system where dredging is frequently performed, this represents a considerable fraction of the allochthonous nitrogen.
5. Without estimations of predation on worm populations it is impossible to state how much of the worm production is returned to the ecosystem and how much is being recycled within the worm - microbiota - sediment complex.
6. Worm populations exert a strong selective influence on the bacterial populations upon which they feed.

SECTION II

RECOMMENDATIONS

The activity of the benthos in retrieving materials and energy from the sedimentary "sink" in aquatic ecosystems should be assessed in any study of productivity or eutrophication of lakes. The secondary production of worm tissue may reach significant proportions and hence may complement their activities in physical stirring and irrigation of sediment. The activities of benthic animals play a significant role in determining the nature and quantity of bacterial populations in sediment, so that the type and amount of benthos present should be noted in bacteriological surveys of aquatic systems as well as the normal abiotic factors.

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SECTION III

INTRODUCTION

In the study of eutrophication we are concerned with the circulation of materials and the flow of energy through aquatic systems. While questions of quality, such as an overabundance of particular algae (*Cladophora*) or certain less desirable fish species (Carp), are of importance, they have attracted less attention than questions of quantity of materials and energy which should be susceptible to analysis.

Materials and energy sedimented out to the bottom may be thought of as descending to a "sink" from which return is difficult. Re-suspension of sediment has been long suspected but rarely demonstrated. Chemical diffusion processes at the mud/water interface have received some attention, but the activities of benthic organisms have not often been thought of in terms of regeneration of material and energy from that sink. The irrigation of sediments by worms may be substantial, and will be investigated in the future. In this study we attempted to measure the potential of a worm population as a means of retrieving material and energy from the sink.

When organic matter from primary production (autochthonous) and from external (allochthonous) sources reach the sediments of a lake they are decomposed by bacteria, which themselves form an important component of the diet of many benthic animals (Wavre and Brinkhurst, 1971). This decomposition creates an oxygen demand (the respiration of microbiota and benthic animals plus chemical oxygen demand of allochthonous materials) and this, together with the clogging action of settling and resettling sediment, limits the diversity of the benthic fauna. The distribution and abundance of benthic animals is the best method of detecting the nature, extent and source of pollution in aquatic ecosystems (Hynes, 1960; Brinkhurst, 1969) but beyond this we need to understand the function of benthic organisms in polluted sediments.

Organic additives, nutrients, or heated effluents may increase production in aquatic systems, despite the natural tendency to deposit nutrients in the sediment or to lose them over the outlet. According to widely held beliefs (largely unsubstantiated and based on a largely discredited lake typology system) any quantum addition of organic matter to a lake will "age" it by remaining in circulation. Significant release of phosphates from anaerobic sediments is accepted, so that an eutrophic lake should release nutrients to the water column in late summer and under ice-cover. This is

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presumed to be available for algal growth in the spring by being stored in algal cells during the periods of complete aeration during ice-free cold periods.

Return from the sediment may occur not only by simple chemical exchange, but by the irrigation of sediment by organisms, particularly worms, and also by the build up of tissue by larger organisms that may migrate into or through the water column or be consumed by migratory organisms such as fish.

If sediments are irrigated by worms, the surface may be aerobic but the faeces are derived from the anaerobic layer, so that their deposition on the surface may release nutrients into the water column. So may the water flow drawn through the sediments (provisionally estimated as better than $600 \text{ ml/m}^2/\text{hr}$ by Wood and Brinkhurst, In Mss.). This problem will be the next to receive our attention, but first we attempted to assess the production of worm tissue in Toronto Harbour and in the trophic gradient of the Bay of Quinte (which strongly resembles the whole Lake Erie system of three basins).

To do this we had to study:

Identity of worm species, their distribution and abundance in the habitats, their standing stocks through time, growth and reproduction (production), respiration, egestion, egestion rate, sediments and sedimentation rate, temperature and oxygen characteristics of the environment. Initial studies ~~on the use of selected components~~ of the potential food supply by specific worms have also been attempted, as have studies of flux rates of specific amino acids and sugars between mud, water, microflora and worm populations.

The primary aim of this study was to evaluate THE RECOVERY OF POTENTIAL NUTRIENTS FROM SEDIMENT VIA THE PRODUCTION OF SLUDGE WORM TISSUE and THE RATE OF DESTRUCTION OF ORGANIC MATTER IN SEDIMENT VIA ASSIMILATION BY WORMS (PRODUCTION PLUS RESPIRATION). The aim is clarified in Figs.1-2.

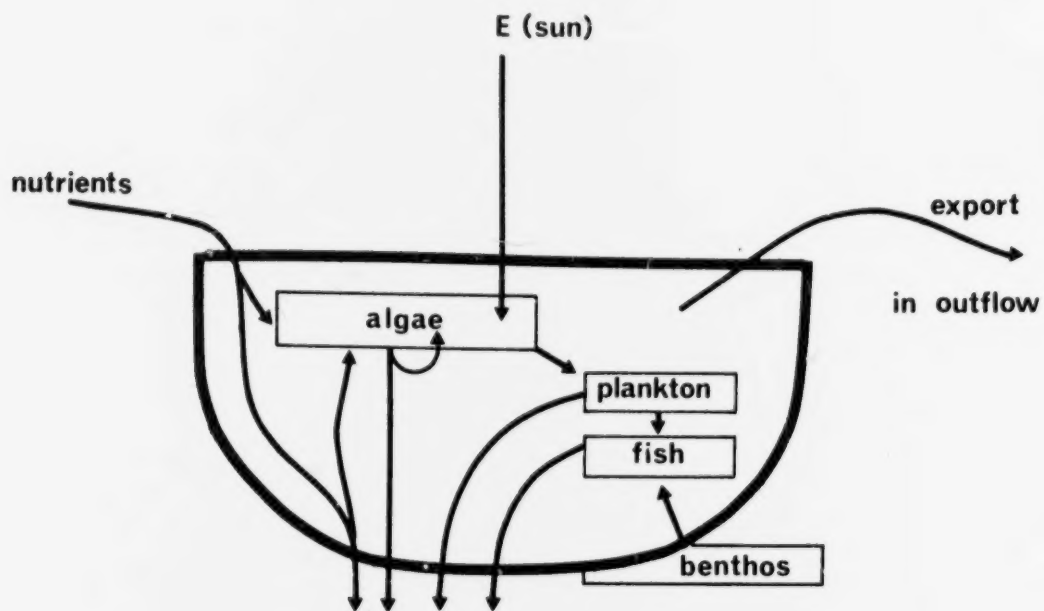


Fig. 1. Common view of nutrient pathways.

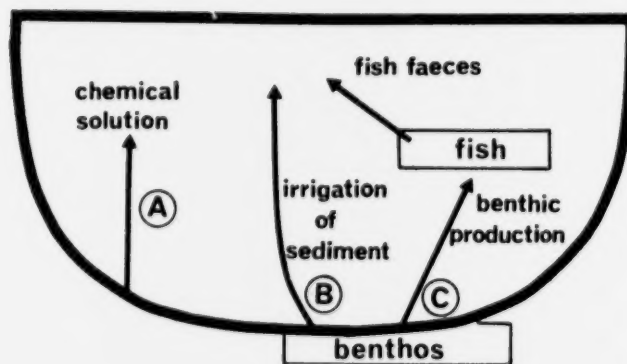


Fig. 2. Potential sources of return from sediment.

SECTION IV

MATERIALS AND METHODS

Toronto Harbour has a surface area of 373 ha and a maximum depth of 12.5 m. The Don River enters the northeast corner of the harbour via the Keating Channel. The harbour is connected to Lake Ontario via two narrow channels (marked by arrows in Fig.3) known simply as the East and the West gaps. The northern and eastern sides are embanked and are used as docks, and the banks are covered with warehouses and industries. The south and west shores are formed by the Toronto islands, between which run a series of lagoons and channels. The islands are mostly used as parkland, with a few residents still occupying the southeast corner. A number of sailing clubs and a small airport form part of the recreational facilities.

The two major sewage works on the Toronto waterfront discharge their effluents on either side of the harbour in Ashridges Bay and Humber Bay. Storm sewers discharge into the harbour along the northern wall, and the Don River carries a heavy load of organic and industrial wastes.

The survey of worms in Toronto Harbour was carried out during May-July 1969, the sampling stations being based on a grid plan. Four samples were taken at each of the 43 stations with a KB corer (5 cm diameter) described elsewhere (Brinkhurst et al., 1969). Samples were screened through narrow mesh screens (0.2 mm opening) and worms were picked using stereo microscopes. Two of the samples were used to determine the dry weight of worms (plus gut content) at each station. Eight of these samples were used to determine the average dry weight of a worm. This value was used to convert dry weight values to standing stocks estimates. The other two samples were used to determine the identity and abundance of each species present. Average values of dry weight for worms minus gut contents for each species were used to obtain calculated dry weight estimates. Some additional data from continuous studies at Hanlan's Point are used in addition to the survey data.

Standing stock data were derived from four core samples collected each month from Hanlan's Point, Toronto Harbour. Samples were sieved using 0.2 mm pore size screens and the worms were identified using a stereo microscope. Worms were held in dechlorinated city tap water at 10°C for 12 hours to allow the guts to clear before being washed and dried in preweighed planchets at 70°C for 12 hours. The planchets were cooled in a dessicator and reweighed on a Cahn electrobalance.

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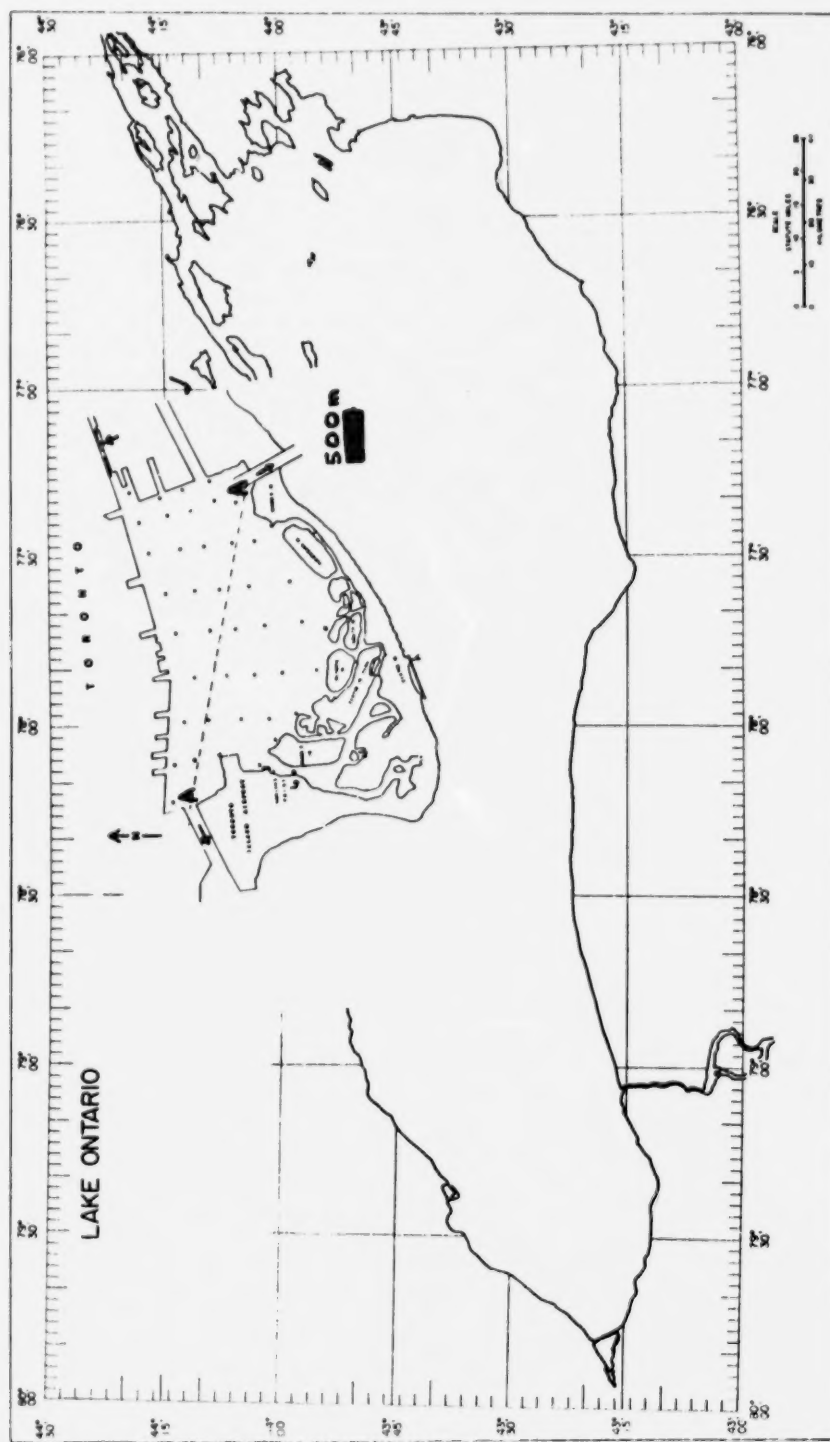


Fig. 1. Sampling stations in Toronto Harbour, survey dated May-July, 1969.

Sediment analyses were made on samples dried at 60°C prior to analysis. Percentage organic matter was determined by igniting samples in preweighed crucibles at 500°C for 1 hour. The cool dry crucibles were then reweighed. Caloric content was determined by wet oxidation by the method described by Hughes (1969). Attempts at microbomb determination of caloric content were abandoned after trying several ways of combining the mud with oil or placing it in paper cups to obtain complete combustion. Nitrogen content was determined by a semi-micro Kjeldahl technique based in methods described by Jackson (1958), the American Instrument Co. (1959), and the Association of Official Agricultural Chemists (1965). Material of known weight (100-300 mg) was kept in a 100 ml flask to which was added 6 ml 36N H₂SO₄ containing 0.3 g salicylic acid. Samples were held overnight, and then digested with about 0.75 g Na₂S₂O₃·5H₂O slowly heated for 5 minutes. The heat was turned off and 0.4 g red HgO, 2.0 g K₂SO₄ and 2 glass beads were added. The flasks were gently reheated until frothing ceased, when the temperature was slowly raised and digestion completed. Steam distillation was carried out after adding 15 ml 50% NaOH containing 5% Na₂S₂O₃·5H₂O. Titration was performed with 0.005 N HCl.

Temperature and oxygen values were obtained with a Mackereth Oxygen Probe (Lakes Instruments Co. Ambleside, Westmorland).

Worms used in respiration studies were also obtained from Hanlan's Point, Toronto Harbour, where the water is 4-5 m deep. Samples were taken with an Ekman grab and sieved in the field in a wash bucket with an 0.5 mm pore size, thus eliminating the smallest worms. The residue was placed in a bucket with some lake water, and a number of such buckets were returned to the laboratory, where they were stored in environmental rooms at 5°C, 10°C, 15°C, and 20°C for at least 3 weeks prior to use. Worms were then sorted with pasteur pipettes from sub-samples sieved through fine screens (0.25 mm pore size) and were identified under a stereo microscope. One hundred and fifty worms of each species were identified, placed in a tube with some sediment and water, and returned to the environmental room for 12 hours. The density used (108,000/m²) was equivalent to that at the sampling site.

The average dry weight of T. tubifex and L. hoffmeisteri used in these studies was high (about 0.4 mg per worm as opposed to 0.06 and 0.2 mg respectively for field populations). All available specimens of the scarcer P. multisetosus were used so that their average dry weight (0.1 mg) was the same as that derived from field populations. Age of worms cannot be established.

The worms were washed in dechlorinated tap water before being introduced into glass vessels containing about 20 ml washed sand (0.25 mm particle size) and 120 ml water, and these respiratory chambers were closed by inserting YSI 5420A self-stirring BOD probes into the neck. The probes were cleaned frequently and the membranes were replaced at least once a week. In each experiment 4 such chambers were prepared, one without worms being used as a control.

Prior to the introduction of the worms, the chambers were placed in a beaker full of dechlorinated tap water covered with foil, and sterilized in an autoclave for 15 minutes. After cooling, the vessels were placed in a constant temperature bath at the experimental temperature, and aerators were introduced for a minimum of 12 hours to ensure that the oxygen saturation remained above 85%. The vessels were eventually placed in an enclosed water bath connected to a Haake constant temperature circulator. A BOD probe was introduced into the neck of each chamber and the system was allowed to stabilize for 2 hours prior to the introduction of the worms. A small piece of stramin was placed over the sand after the worms had burrowed into it, and this was held down by a number of glass beads. This was to prevent the stirrer from agitating the sand too violently. The worms protruded their posterior ends through the stramin into the water in the normal manner. An initial reading on the YSI dissolved oxygen water was taken 10 minutes after the worms were introduced, and then again after 2 hours. The control values were subtracted from the gross respiration data to obtain net respiration values. Short-term experiments were used in order to eliminate problems due to recolonization of sterile sediment by bacteria from the worm faeces. Continuous records were not kept because of the problem of continually agitating the worms - every precaution being used to keep conditions as natural as possible.

The main series of experiments was carried out using 150 worms of a single species in each respiratory chamber, the fourth chamber lacking worms being used as a control. Once oxygen levels had been determined (after 10 minutes and 2 hours) the worms were retrieved and held overnight in sieved mud. The same individuals were then used again the next day in the second half of each experiment, this time equal numbers of each of the three species being mixed together and introduced into each chamber.

At the end of each run, the mixed worms were re-identified. The worms of each species from each respiratory chamber were placed in separate test tubes in water and were held overnight at 10°C to allow

the gut contents to be voided. They were then dried on planchets at 70°C for 12 hours and weighed on a Cahn electrobalance. The dry weights obtained could then be summed a) according to species in order to determine the rates of oxygen utilization in pure culture and b) according to respiratory chamber occupied by a mixture of all three species in the second half of each experiment.

Each run (separate then mixed cultures) was repeated 3 times at each temperature used (5°C, 10°C, 15°C, and 20°C). Hence, at the end of this series of experiments 3 determinations of oxygen uptake by each of 3 species and 9 determinations of each mixed culture had been obtained at each of 4 temperatures. Each determination is, however, an average rate for the duration of the experiment.

The results were calculated as follows. In order to compare the respiratory rates of one species with another, the rate determinations from each of the 3 experimental runs at a given temperature were simply averaged. The respiratory rates of mixed cultures were obtained in a slightly different manner, because there are 3 replicates of this arrangement in each experimental run. The total volume of oxygen used in all three chambers of a run was divided by the total weight of worms to obtain an overall value for respiratory rate. An average of the 3 determinations at each temperature was then calculated. This was then compared with a value for the respiratory rate of worms held in pure culture derived as follows. The total oxygen consumption in all 3 bottles (one containing *T. tubifex*, one with *L. hoffmeisteri* and the third with *P. multi-setosus*) was divided by the total weight of worms used in the experiment (the same weight used to calculate the respiration rate of mixed cultures in the same run). The 3 values were then averaged. This procedure is described in detail as it is possible to average the values in other ways. Examination of the tabulated results should further clarify the procedure adopted.

As the results obtained by mixing the worms could be attributed to the degree of handling or starvation, the process was repeated at 10°C but the respiratory rate of mixed cultures was determined before that of the pure cultures. Once again the experiment was repeated 3 times, yielding 9 values for mixed cultures, and 3 values for each of the 3 species isolated in pure culture.

By mixing 50 specimens of 3 species in a single chamber, the actual population density of any one species per chamber has been reduced to 1/3 of its density in pure culture despite the fact that the

population density of all worms remained at 150 per chamber. The possible effect of this was determined by comparing the respiratory rate for each species at densities of 150 and 50 per chamber, again at 10°C and with 3 replicates.

Worms used for growth studies were also collected from Hanlan's Point. Starved worms were wet-weighted on a Cahn electrobalance in a fully saturated atmosphere prior to and at the conclusion of each experiment. Dry weight was taken to be 16% of wet weight (Brinkhurst, 1970).

Cultures were maintained for 6 months because growth studies based on cultures maintained for between 9 and 20 days proved unsatisfactory because of weight losses or slight weight gains rather like those demonstrated by Appleby and Brinkhurst (1970).

The worms were maintained in pure culture and also in every possible combination of pairs as well as mixtures of all 3 species. In pure culture 18 worms per vessel were used, 9 of each species were used in paired situations, 6 of each species where all 3 were present, densities far short of those found in Toronto Harbour.

The sediment used was autoclaved for 20 minutes at 110°C and 20 psi to eliminate small worms and cocoons that would pass through fine screens. After cooling, the sediment was inoculated with an 18 hr stock culture of bacteria in nutrient broth. The culture contained 5 of the forms found in Toronto Harbour (Wavre and Brinkhurst, 1971) i.e. Flavobacterium sp. Bacillus cereus, B. mycoides, Pseudomonas fluorescens and a Micrococcus sp. The sediment once inoculated was held for 48 hr at 20°C prior to the introduction of the worms. About 400 ml of wet mud was placed in each 600 ml plastic box into which 18 worms were then introduced. The boxes were placed in wooden containers at random, and dechlorinated city water (from Lake Ontario) was allowed to flow through the system continuously, the water level being maintained at twice the height of the plastic boxes. Temperature records were kept daily, and were roughly comparable with the field temperatures (mean temperature 10.9°C in the laboratory, 9.7°C for the same period in the field in 1969 and 1970). A total of 5 replicates of each combination was used, and as there are 7 combinations possible, a total of 35 plastic boxes was used. At the end of 6 months (July 12, 1970 to January, 1971) the worms were retrieved by washing the mud through a 0.25 mm screen. They were identified, counted, dried, and weighed.

Analysis of variance was performed using the method described by Snedecor and Cochran (1968).

The collection of faeces was made using the Alsterberg inverted-culture technique in which worms are placed in sediment in small vials which are then covered with burlap (Fig.4). Once the worms protrude through the fabric, the vial may be inverted over a collecting vessel into which faeces fall (Appleby and Brinkhurst, 1970).

Worms were weighed with the guts empty prior to each experiment using a Cahn electrobalance, and 3 samples of worms were used to determine wet weight/dry weight ratios at the beginning of each experiment. These values were used to determine the dry weights of worms used in each vial. Unless otherwise stated the mud used in the vials was sieved through a No.60 screen (250 μ pore size) and allowed to settle overnight. Once in the partially filled vials, the worms were covered with a little of the silt and a layer of wet cotton was added, followed by a piece of stramin. The vials were kept upright for two days, and then inverted for two days before faecal samples were taken. The faeces were collected on preweighed planchets in glass chambers (60 x 35 mm), the collecting funnels penetrating neoprene stoppers that isolated the collecting chambers from the surrounding aquarium tank. The chambers were filled with 50 ml distilled water, to which was added 300 mg NaCl. Once this had dissolved, 0.3 ml of an iodine solution was introduced slowly, just above the planchet, so that a discrete layer formed below the salt solution. The salt and iodine removed from the stem of the collecting funnel, and the system was topped up with distilled water. These precautions were taken to minimize the development of bacteria on the faeces.

One such set-up was placed beneath each experiment vial for 48 hours at a time. After the first time period the faeces were weighed and used for the determination of % organic matter. After the second interval the fresh faeces were used to obtain nitrogen content, the third set being used for calorific values. The same sequence of samples was taken a second time with the same set of experimental vials, sometimes more often. Sometimes samples had to be pooled to obtain sufficient material for analysis. Control vials full of sediment were kept in the aquaria along with the experimental chambers at all times in order to determine changes in the parameters measured brought about by the microflora alone.

An initial experiment was run at 5°C and a subsequent experiment was run at 10°C, 15°C and 20°C using one stock of sediment which was made up separately to that used at 5°C. In addition to the effect of temperature, preliminary assessment of the effect of particle size was made at 5°C alone, using mud passed through No.60,

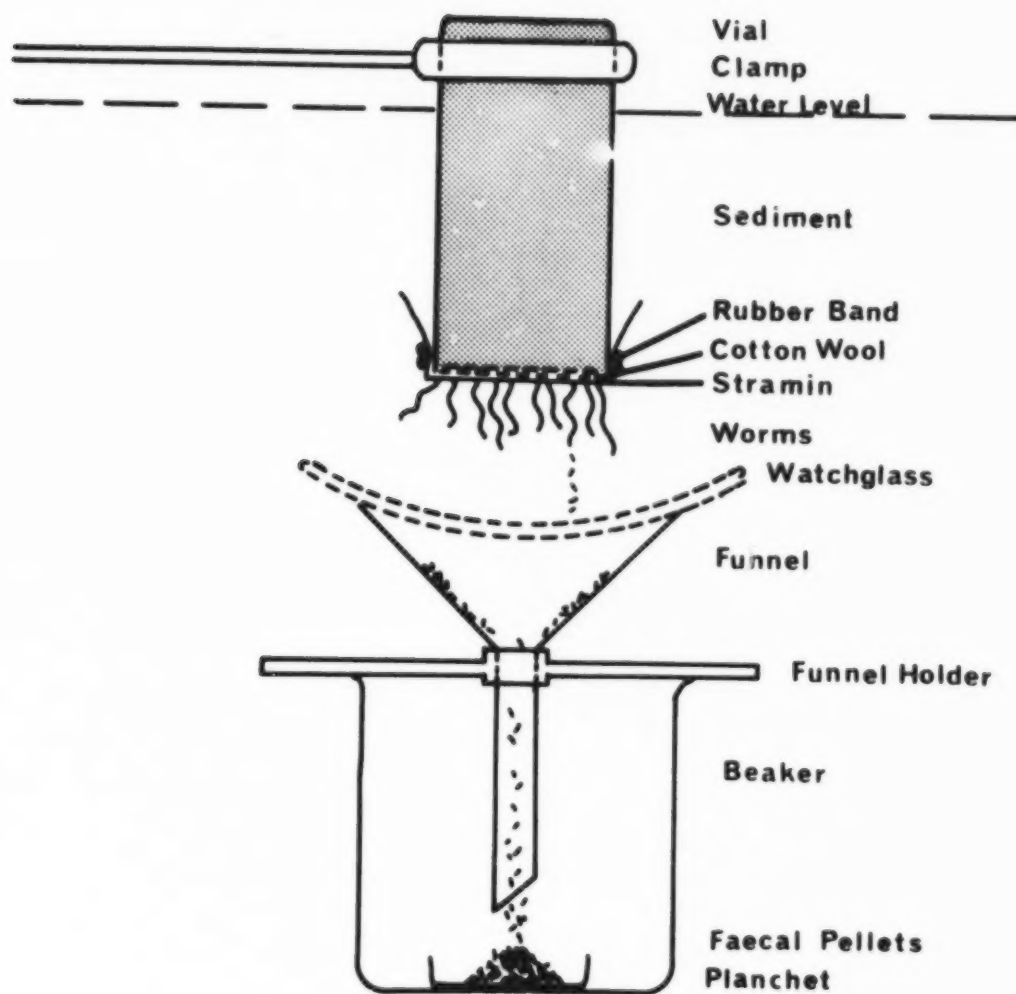


Fig. 4. Apparatus for collecting faeces. The whole assemblage is kept in an aquarium.

120, and 230 screens (250, 120 and 63 μ respectively).

In a final series, run at 10°C for 24 and 48 hours, defecation rates were determined for fifteen worms of each species in pure culture, and a total of fifteen mixed worms (five of each species).

Calorific values of the three species of worms were determined by igniting 5 large samples of worms of each species in Phillipson microbomb (Gentry Wiegert) calorimeter. An oxycaloric coefficient of 4.825 kcal/l O₂ was used to convert respiration data (Brody, 1945). The worms were held in dechlorinated tap water overnight before being dried at 70°C for 20 hours.

A pellet press was used to compact the worm tissue. Five replicates were obtained. Ash-free dry weights were determined by ignition at 500°C for 20 minutes. The ash content of worms was found to be 8.5, 9.7 and 23.6% for T. tubifex, L. hoffmeisteri and P. multisetosus.

SECTION V

TORONTO HARBOUR STUDIES

The study area consists of a busy commercial harbour on the St. Lawrence Great Lakes at Toronto, Ontario (Canada). The seaport is enclosed by a chain of islands and sand bars used as a park and an airport with a few residents at the southeast end. The location is illustrated in Fig.3.

Some of the environmental variables were described by Bennett (1970), Brydges (1970) and Michalski (1970). Temperature and oxygen records are presented in Appendix 1, data on nitrogen and percent organic matter of sediments in Appendix 2.

The maximum depth of the harbour is 12.5 m (41 ft) the surface area approximately 373 ha (920 acres). The Don River enters at the north east corner, bringing with it a considerable amount of domestic and industrial waste. Wastes also enter the harbour from the storm sewer outfalls along the northern wall. The ships in the harbour and the residents plus the huge transient population of day trippers in summer add to the pollution of the environment. Toronto's major sewer outfalls lie outside the harbour proper and are unlikely to affect conditions within it. Coliform values north of the line A-A Fig.3 were greater than 2400/100 ml, below it in excess of 1000/100 ml ($\bar{x}$ 2220) in December 1967 (O.W.R.C. data).

The distribution and abundance of sludge worms (Tubificidae) has been described by Brinkhurst (1970). The data presented here are in the form of maps indicating generalized zones (Figs.5-10). Most tubificids are found in the northeast and northwest corners, least in the island lagoon area. The commonest species are Tubifex tubifex, Limnodrilus hoffmeisteri and Pelosclex multisetosus (Table 1) but there is a gradual change from this pollution tolerant complex at the mouth of the Don River, to a mixture of eutrophic lake species in Zone IV, where Aulodrilus and Potamothrix species are found. Data on the distribution of the three commonest species are presented in Table 2. The totals derived from counting identified worms from paired samples are very close to those calculated from dry weights of all worms in the second set of paired samples from the same station. Samples taken at one point during the period 2 a.m. to 10 p.m. on a single day revealed no diurnal changes in depth - distribution of worms in the sediment (Table 3). The single period that seems to suggest diurnal changes (2 p.m. values) is that when most samples have been taken over extended periods, when the vertical distribution pattern is similar to that shown in the rest of Table 3 (Brinkhurst et al. 1969).

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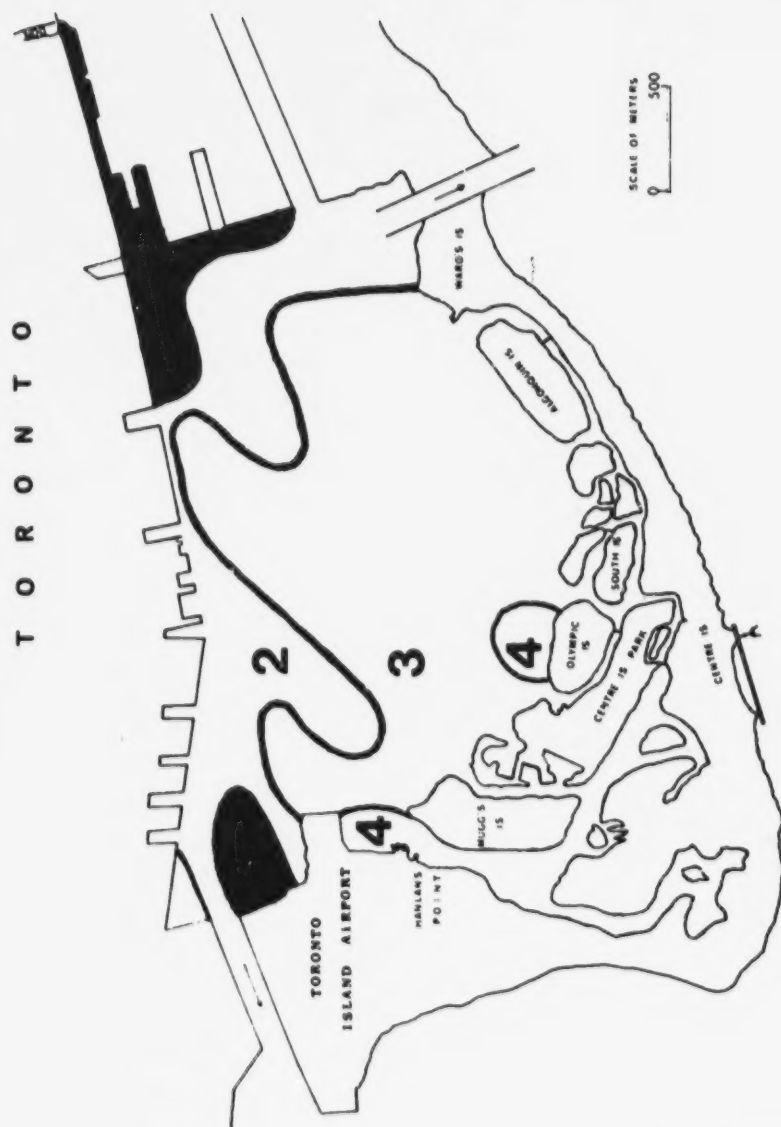


Fig. 5. Abundance ($\text{no.}/\text{m}^2$) of tubificids in Toronto Harbour. Zone 1 (solid), $>125,000$; zone 2, $75,000-125,000$; zone 3, $25,000-75,000$; zone 4, $>25,000$.

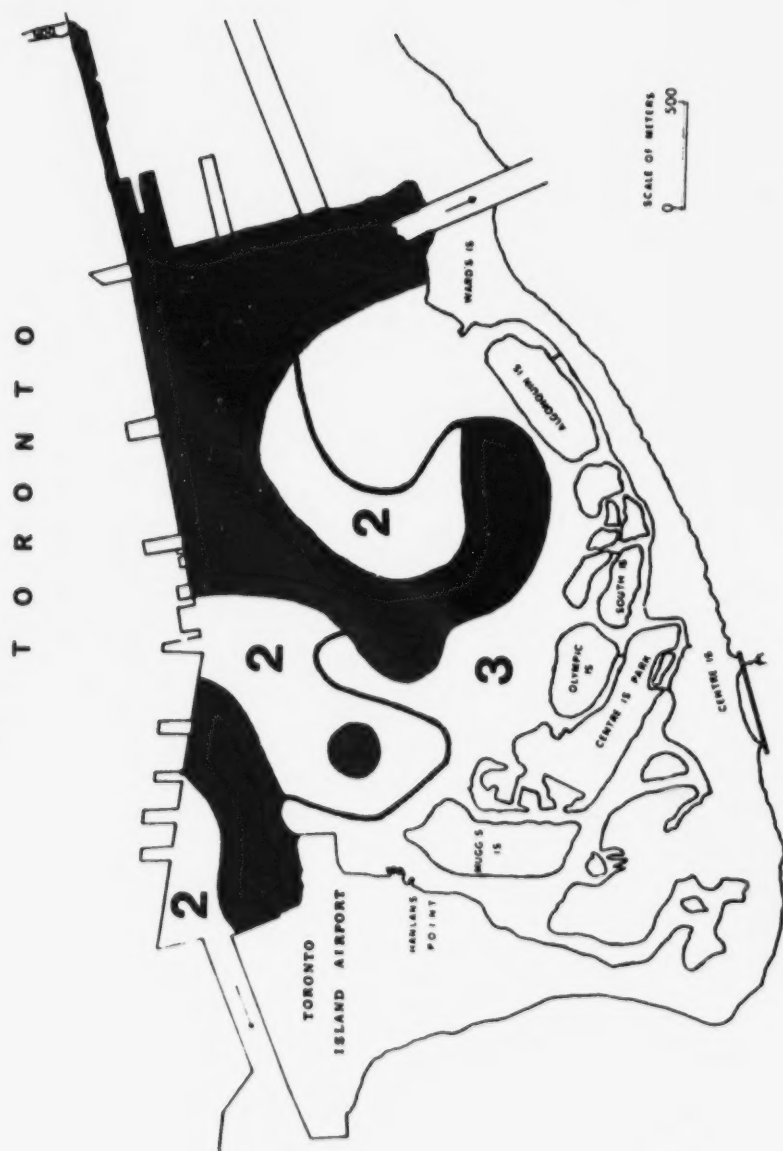


Fig. 6. Abundance of Tubifex tubifex in Toronto Harbour as percentage of all tubificids. Zone 1 (solid), >50%; zone 2, 25-50%; zone 3, <25%.

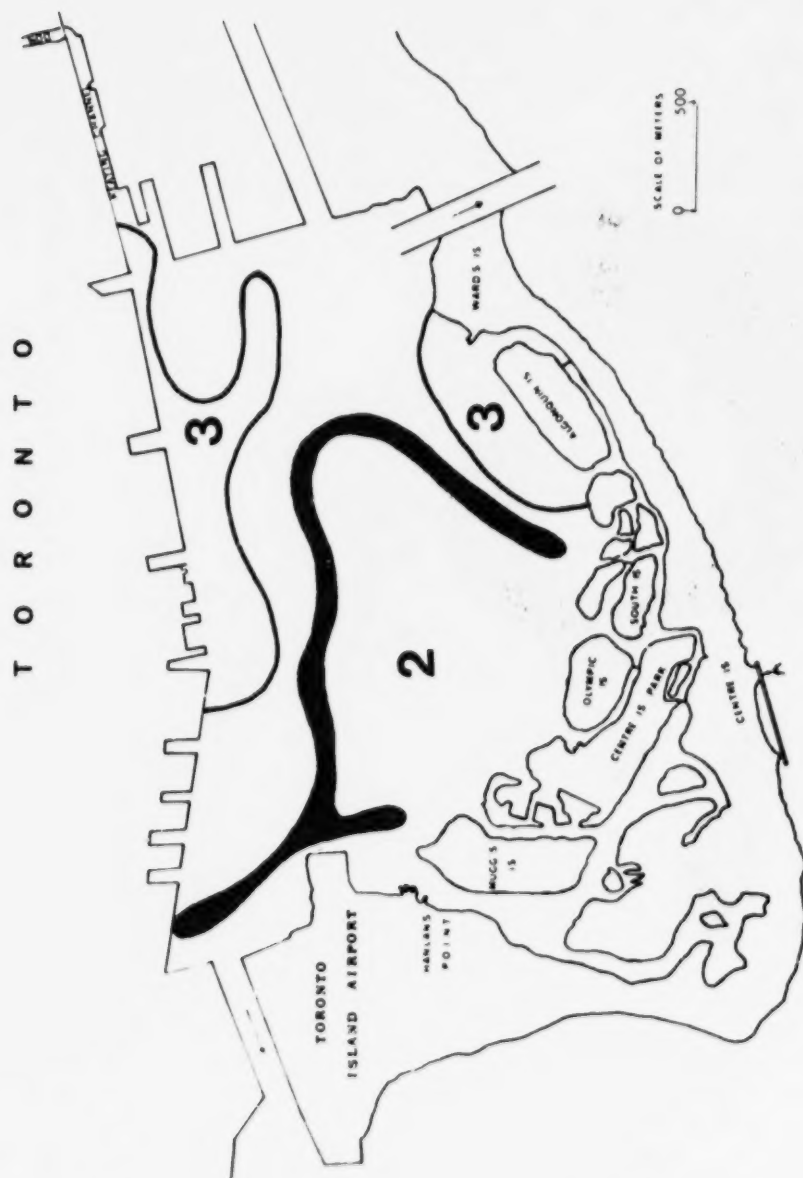


Fig. 7. Abundance of Limnodrilus hoffmeisteri in Toronto Harbour as percentage of all tubificids.
 Zone 1 (solid), >50%; zone 2, 25-50%, zone 3, <25%.

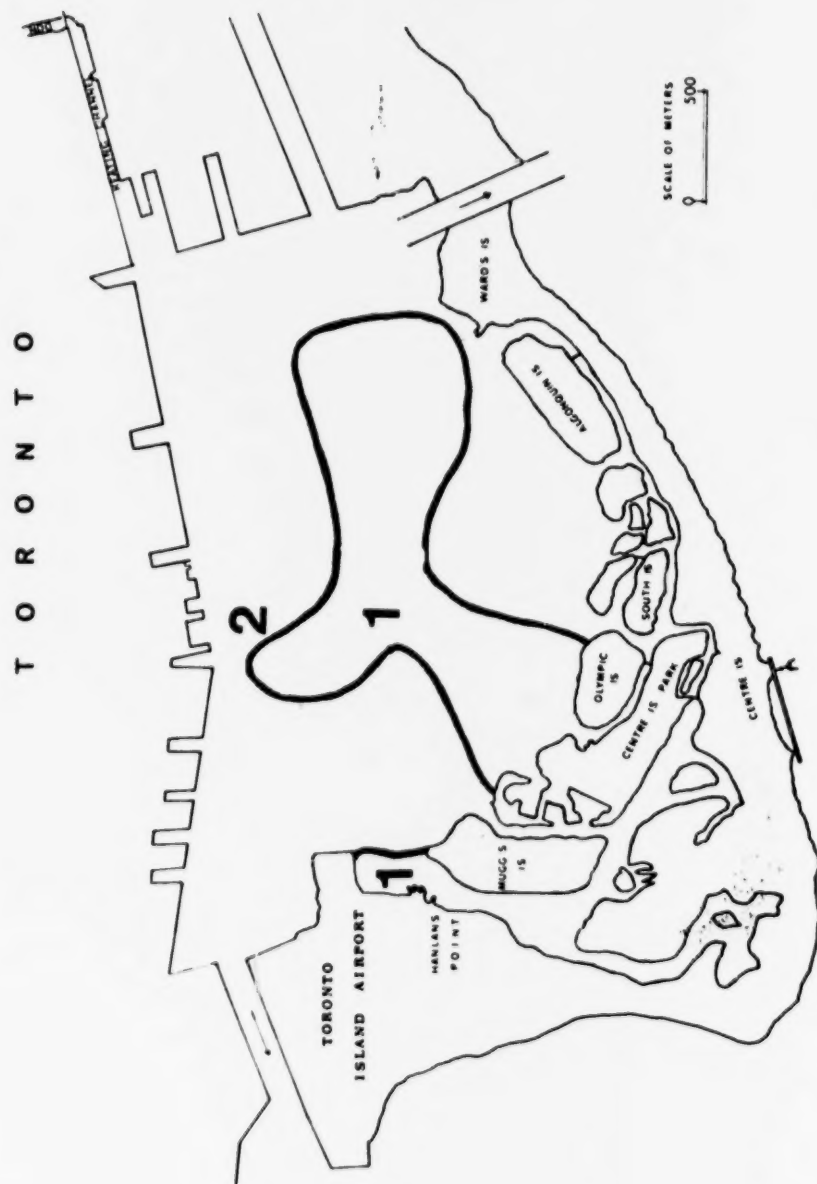


Fig. 8. Abundance of *Peloscolex multisetosus* in Toronto Harbour as percentage of all tubificids.
Zone 1, >25%; zone 2, <25%.

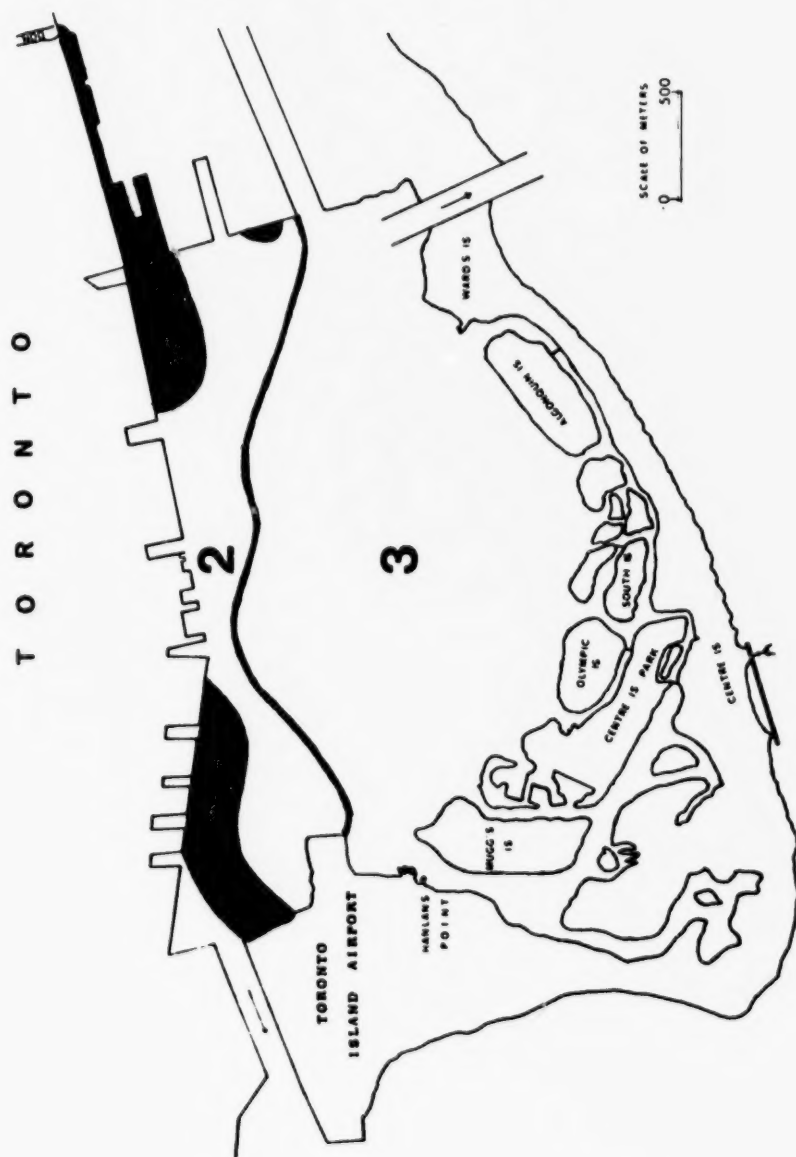


Fig. 9. Abundance ($\text{no.}/\text{m}^2$) of Limnodrilus udekemianus in Toronto Harbour. Zone 1 (solid), >5000 (mean, 9500); zone 2, 1000-5000 (mean, 2500); zone 3, 0-500 (mean, 360).

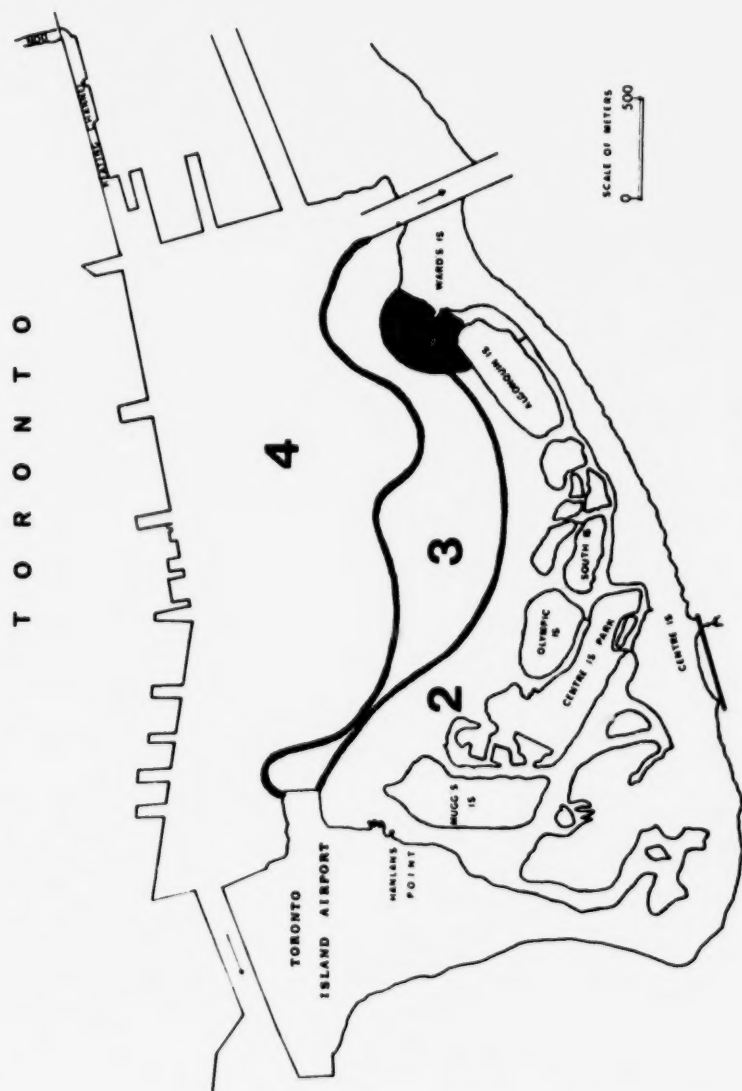


Fig. 10. Abundance (no./m²) of Aulodrilus plurisetus and Potamothrix vej dovskiy in Toronto Harbour.

A. plurisetus: zone 1 (solid), 9250; zone 2, 750-3750 (mean, 2000); zone 3, 250-750 (mean, 350); zone 4, absent. P. vej dovskiy: zones 1 and 2 present (mean, 800); zones 3 and 4, absent.

Table 1. Abundance of tubificid species in Toronto Harbour.

Number	Tubifex tubifex	Limnodrilus hoffmeisteri	Pelosclex multisetosus	Other species	Total
(thousands/m ²)	39	26	13	6	84
%	47	31	15	7	100

Table 2. Data on the distribution and abundance of the three most abundant tubificids (average number/m², percent, and dry weight in g/m²) for each of the four zones identified in Fig.1, and for Hanlan's Point, Toronto Harbour. T = Tubifex tubifex; L = Limnodrilus hoffmeisteri; P = Pelosciolex multisetosus.

		Zone				
		1	2	3	4	Hanlan's Point
No. stations		8	11	22	2	1
Avg no./m ² (thousands)	T	115	55	18	3	12
	L	58	33	21	8	16
	P	24	16	12	12	2
	Σ	197	104	51	23	30
Avg no./m ² (thousands) calculated from dry weight plus gut content	Σ	194	106	59	19	-
%	T	58	53	36	14	40
	L	30	31	41	33	53
	P	12	16	23	52	7
Avg dry weight (g/m ²) calculated for worms minus gut content	T	22.9	11.0	3.7	0.7	2.4
	L	17.5	9.8	6.3	2.4	4.9
	P	2.3	1.6	1.1	1.2	0.2
	Σ	42.7	22.4	11.1	4.3	7.5
Avg dry weight (g/m ²) of worms plus gut contents (all species)		98.5	48.0	26.5	9.0	-
Ratio dry weight plus gut content/ dry weight without gut content		2.3:1	2.1:1	2.4:1	2.1:1	-

Table 3. Vertical distribution of worms in sediment during one diurnal period. Numbers are given per core sample; numbers per square meter are approximately 500 times greater.

Depth in core (cm)	Time					
	2 AM	6 AM	10 AM	2 PM	6 PM	10 PM
0-2	47	91	56	10	60	41
2-4	14	40	28	14	36	33
4-6	7	21	9	11	11	12
6-8	2	6	7	4	2	4
8-10	0	4	4	1	5	2
10-12	12	2	0	2	1	5

The distribution and abundance of tubificid species in Toronto Harbour clearly demonstrates the polluting effect of the Don River, which is still subject to intense stress despite considerable improvement over the last few years. This fact is reinforced by a consideration of the rest of the fauna. Over nearly all of the open harbour and in the docks themselves the tubificids are the entire fauna. Among the island lagoons Sphaerium, Pisidium, Valvata, Chironomus, Orconectes, Asellus, Gammarus, Caenis, Trichorythodes, Ischnura, Chromagrion, Glossiphonia, Helobdella and a number of other genera were found in small numbers. (Operation Sludgeworm - Opportunities for Youth 1971 - Final Report).

The detailed distribution of tubificid species confirms earlier reports of the relationship between sludge worms and pollution documented elsewhere (Brinkhurst and Jamieson, 1971).

Both numbers and weights of worms varied through quite wide limits in 1970, although the plots of the logs of values obtained demonstrate no seasonal trends (Figs.11-12). An attempt to demonstrate breeding periods by reference to mean weights failed, and the supposed upward trends in mean weight revealed during 1970 (Fig. 13) were regarded as spurious despite the statistically significant differences in slopes of the lines as subsequent data failed to conform to this pattern. Steady changes of slope like this with time could relate to long-term changes in conditions in the locality, in which case a similar trend from Zone I to Zone IV of Toronto Harbour (Fig.5) might be demonstrable. The results of such a test are reproduced in Table 4, and give little credence to the idea.

Tubificid worms do not all reproduce at a given time for a given species, or at a fixed time in any one habitat. Reproduction may occur very rapidly after intermittent pollution stress, but no discrete cohorts may be detected, and the life-span of a worm in the field is difficult to determine. Worms may live two years, breeding mostly in their second year (Brinkhurst and Jamieson, 1971) but in polluted localities, such as Toronto Harbour, life histories are very difficult to establish. While worms may reach a peak biomass in August with a minimum in December, numbers appear maximal in March and September, but these could be sampling artifacts only to be overcome by intensive sampling programmes beyond our resources. For studies on production we will assume constant standing stocks equal to the means derived from the 1970 data.

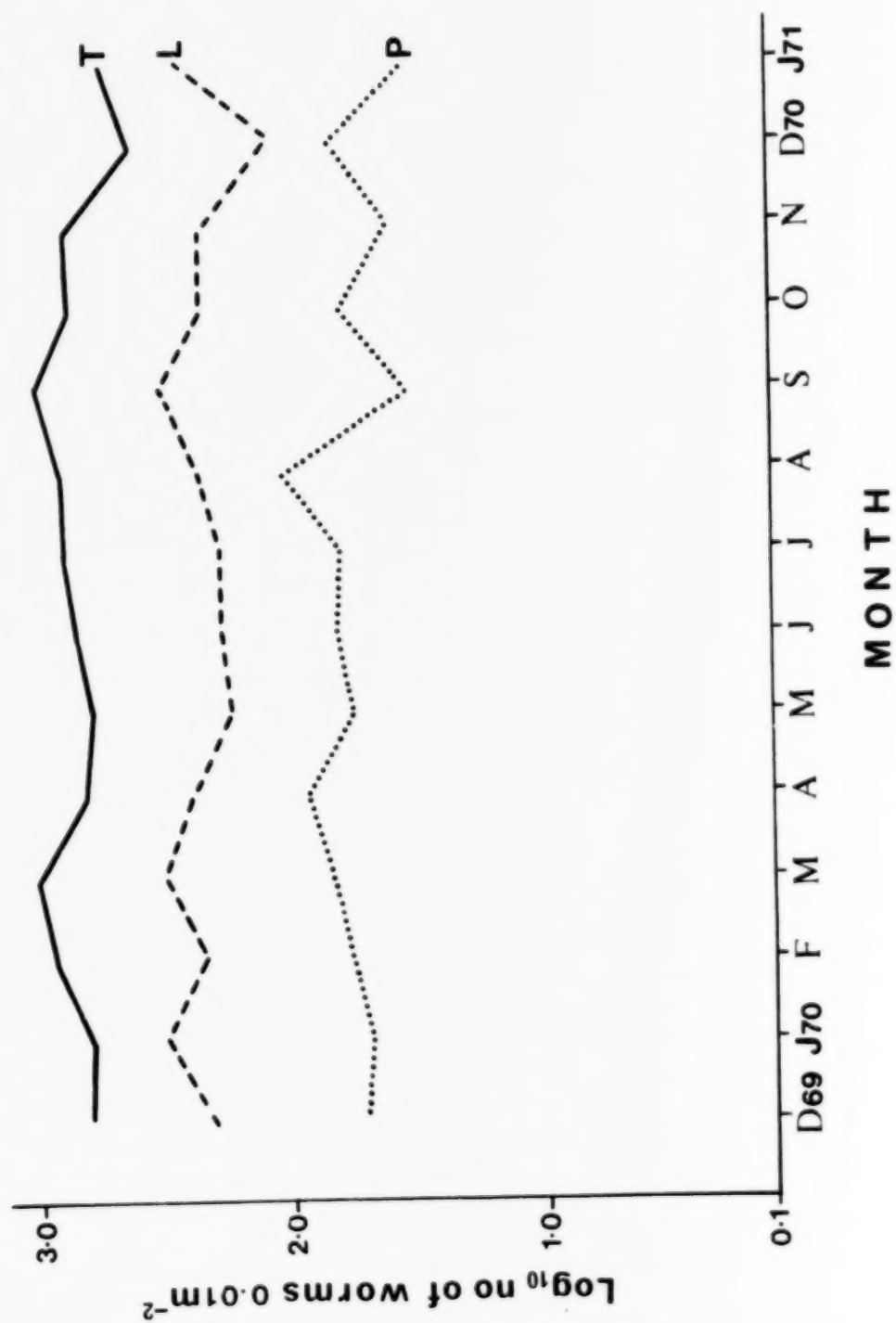


Fig. 11. Seasonal abundance of three worm species.

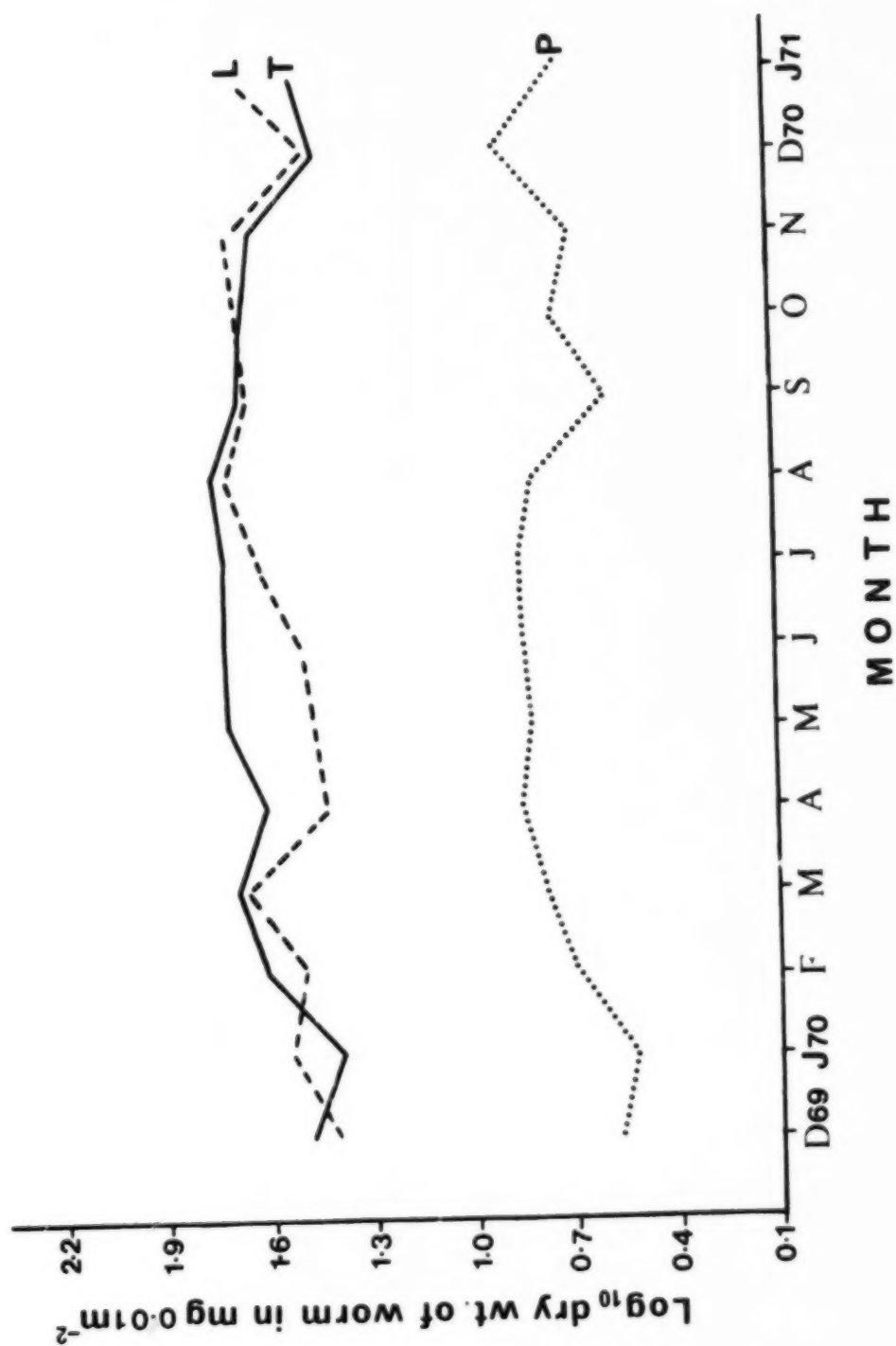


Fig. 12. Seasonal changes in biomass of three worm species.

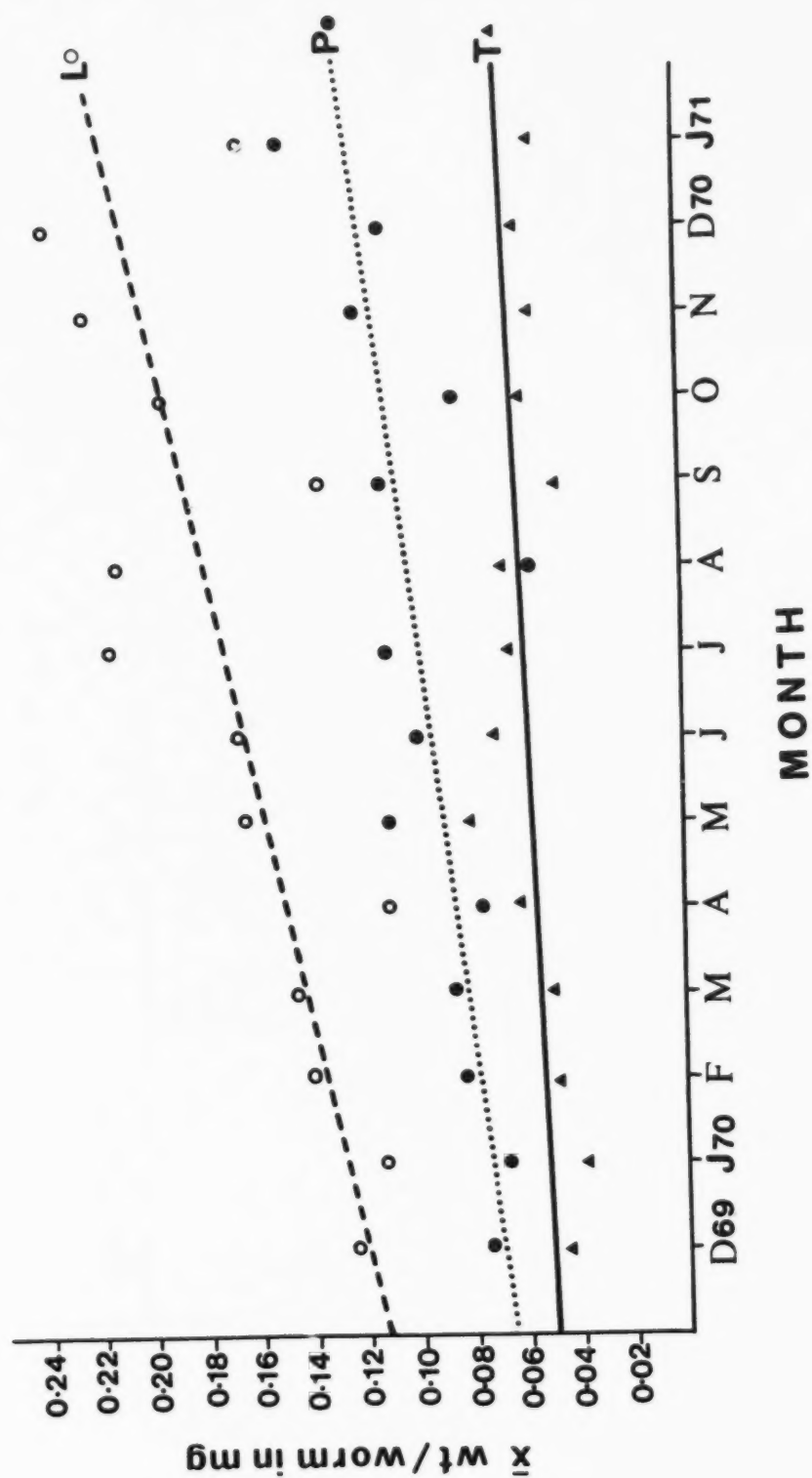


Fig. 13. Supposed seasonal changes in mean weight of worms.

Table 4. Average dry weight in mg per worm at the most polluted (Zone 1) and least polluted (Zone 4) parts of Toronto Harbour, July 1971.

	<u>Tubifex</u> <u>tubifex</u>	<u>Limnodrilus</u> <u>hoffmeisteri</u>	<u>Pelosclex</u> <u>multisetosus</u>
Zone 1	0.147	0.209	0.093
Zone 4	0.100	0.208	0.094

SECTION VI

PRODUCTION STUDIES

As individual worms cannot be aged, and there are no clear cohorts in the population structure of worms, production studies have to be based on the method derived by Ivlev (1939). In this approach laboratory studies of ingestion, growth, respiration, egestion, excretion and secretion are carried out in order to balance the equation:

$$\text{Ingestion} = \text{Production} + \text{Respiration} + \text{Egestion} + \text{Excretion} + \text{Secretion}$$

It is usually difficult to obtain ingestion values, so that growth (including reproduction) respiration and solid egestion are measured in calorific terms and the formula is solved for ingestion assuming that fluid excreta and secretions are trivial. Most of the assumptions have not been fully tested until recently (Hargrave, 1971). In our study we attempted to evaluate each parameter (other than secretions and fluid excreta) for three species reared in isolation and in mixed cultures.

Respiration

The rate of oxygen consumption in $\mu\text{l/mg}$ dry weight/hr of the 3 species when measured alone varies in relation to temperature as demonstrated in Table 5. A two-way analysis of variance demonstrated significant differences due to temperature ($F = 11.02$ on 3 and 24 d.f., $P < 0.01$). As there were significant differences between species ($F = 4.85$ on 2 and 24 d.f.) a one-way analysis was performed in order to determine which differences between species were significant. This showed a significant difference between P. multisetosus and L. hoffmeisteri at the 5% level, but none between P. multisetosus and T. tubifex or L. hoffmeisteri and T. tubifex.

The data for separate species compared to that from subsequently mixed worm cultures again varied with temperature, of course, but the data from mixed cultures differed from those obtained from pure culture (Table 6). A two-way analysis of variance indicated a significant effect of temperature ($F = 37.18$ on 3 and 16 d.f., $P < 0.01$) and also a significant difference between separate and mixed cultures ($F = 14.77$ on 1 and 16 d.f., $P < 0.01$). The interaction of temperature vs separate or mixed cultures was not significant.

A Duncan multiple range test (Steel and Torrie, 1960) applied to the interactions between temperatures for both separate and mixed cultures demonstrated a significant difference ($P < 0.01$) between results obtained at 20°C and those at all other temperatures in both separate and mixed cultures. A significant distinction could be made ($P < 0.05$)

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Table 5. Rates of oxygen consumption in $\mu\text{l}/\text{mg}$ dry weight/hr for 3 tubificid species at 4 temperatures (T = T. tubifex, L = L. hoffmeisteri, P = P. multisetosus).

Temperature	Expt No	Total dry weight (mg)			Oxygen consumed (μl/hr)			Respiration rate (μl/mg/hr)			
		T	L	P	T	L	P	T	L	P	
5C	1	47.2	72.9	22.2	14.7	12.3	07.7	0.31	0.17	0.35	
	2	81.1	68.3	16.7	15.1	13.7	03.5	0.19	0.20	0.21	
	3	71.4	75.5	13.4	15.8	13.0	08.1	0.22	0.17	0.60	
								$\bar{x}$	0.24	0.18	0.39
10C	1	39.2	63.0	18.2	11.2	14.0	08.1	0.29	0.22	0.44	
	2	51.1	71.3	18.7	08.4	13.3	03.5	0.17	0.19	0.19	
	3	77.1	66.2	19.6	14.7	14.0	08.1	0.19	0.21	0.41	
								$\bar{x}$	0.21	0.21	0.35
15C	1	41.6	47.1	15.4	18.2	16.8	07.0	0.44	0.36	0.45	
	2	61.7	76.9	16.9	24.5	17.5	04.6	0.40	0.23	0.27	
	3	66.4	55.8	15.2	23.5	18.9	08.1	0.35	0.34	0.53	
								$\bar{x}$	0.40	0.31	0.42
20C	1	55.4	52.9	19.2	23.5	19.2	13.0	0.42	0.36	0.67	
	2	55.3	60.7	14.9	31.5	29.1	05.3	0.57	0.48	0.35	
	3	65.5	51.9	12.6	35.0	30.5	08.8	0.54	0.59	0.70	
								$\bar{x}$	0.51	0.48	0.56

Table 6. Rates of oxygen consumption in $\mu\text{l}/\text{mg}/\text{hr}$ for 3 tubificid species at 4 temperatures determined in pure culture and mixed culture (see text for details of computing data from pure cultures).

Temperature	Expt No	Total dry weight (mg)	Σ oxygen used by 3 species in pure culture ($\mu\text{l}/\text{hr}$)	Σ oxygen used in mixed culture of 3 species ($\mu\text{l}/\text{hr}$)	respiratory rates of 3 species in pure culture ($\mu\text{l}/\text{mg}/\text{hr}$)	Overall respiratory rate of 3 species in mixed culture ($\mu\text{l}/\text{mg}/\text{hr}$)
5C	1	142.2	34.7	22.4	0.24	0.16
	2	166.1	32.2	12.0	0.19	0.12
	3	160.3	36.8	22.8	$\bar{x}$ 0.23 0.22	$\bar{x}$ 0.14 0.14
10C	1	120.5	33.3	21.0	0.28	0.17
	2	141.1	25.2	17.5	0.18	0.12
	3	162.9	36.8	25.9	$\bar{x}$ 0.23 0.23	$\bar{x}$ 0.16 0.15
15C	1	104.1	42.0	33.3	0.40	0.32
	2	155.4	46.6	28.0	0.30	0.18
	3	139.2	50.4	34.0	$\bar{x}$ 0.36 0.36	$\bar{x}$ 0.24 0.25
20C	1	127.5	55.7	45.9	0.44	0.37
	2	130.8	65.8	52.5	0.50	0.40
	3	129.9	74.2	68.3	$\bar{x}$ 0.57 0.50	$\bar{x}$ 0.53 0.43

between 10°C and 15°C in separate cultures only, but all other comparisons showed no significant difference.

When the experimental procedure was reversed (at 10°C) and the species were mixed first and then separated, the results obtained were 0.233 and 0.322 $\mu\text{l}/\text{mg}/\text{hr}$, respectively. Again, the result was highly significant ($F = 184.94$ on 1 and 4 d.f., $P < 0.001$). When two densities were compared (Table 7) no significant differences in respiration rate could be demonstrated.

Growth

The growth of worms over a 6 month period under various conditions is documented in Tables 8 and 9, from which it is clear that growth varies in relation to the presence or absence of other species. An initial consideration of growth in pure culture only shows that, according to a one-way analysis of variance, there is no significant difference in growth between T. tubifex and L. hoffmeisteri, but P. multisetosus grew less than either. T. tubifex grows significantly (at the 5% level) better than P. multisetosus when they are mixed as a pair, and so does L. hoffmeisteri ($P < 0.01$). This result is obtained for the third time when considering the growth of the same pairs when all 3 species are mixed together, but the significance of the result is at the 1% level for both comparisons using P. multisetosus.

Thus far comparisons have been based on cultures initiated with the same number of specimens of each species (18 in pure culture, 9 of each when paired, 6 of each when all species present). Some of the following tests of the growth of single species under a variety of conditions must inevitably involve comparisons based on the results of experiments in which there were differences in the initial numbers used. It would be impossible to create a situation in which the total population density and the population density of each species in separate and mixed cultures remained constant.

The growth of P. multisetosus was not significantly affected by the presence or absence of one or other or both other species, but the growth of the other species was often affected by the presence of this relatively slow-growing species. L. hoffmeisteri grew more in the presence of P. multisetosus than it did alone or with T. tubifex ($P < 0.05$). T. tubifex grew more in the presence of either (or both) other species than when alone ($P < 0.01$). When all 3 species were present T. tubifex grew significantly more than it did in the presence of just L. hoffmeisteri, but the reverse situation (L. hoffmeisteri with T. tubifex vs L. hoffmeisteri with both other species) made no difference to the growth of L. hoffmeisteri. Both species grew as well in the presence of P. multisetosus as they did in the

Table 7. Rate of oxygen consumption in $\mu\text{l}/\text{mg}/\text{hr}$ at 10C of 3 tubificid species at 2 population densities.

Species	Number of worms per test vessel	
	150	50
<u>L. hoffmeisteri</u>	0.24	0.27
<u>T. tubifex</u>	0.31	0.33
<u>P. multisetosus</u>	0.64	0.78

Table 8. Gain in weight of cultures of 3 tubificid species under various conditions as multiples of the original weights.

Culture conditions	<u>L. hoffmeisteri</u>	<u>T. tubifex</u>	<u>P. multisetosus</u>
Pure culture	2.39 7.20 8.02 4.89 <u>2.39</u> $\bar{x}$ 4.98	2.47 1.85 2.74 3.48 <u>4.34</u> $\bar{x}$ 2.98	1.54 1.02 1.00 2.94 <u>1.76</u> $\bar{x}$ 1.65
With <u>L. hoffmeisteri</u>	- - - - -	4.61 5.30 5.80 7.25 <u>6.53</u> $\bar{x}$ 5.90	0.34 0.50 0.88 2.32 <u>3.81</u> $\bar{x}$ 1.57
With <u>T. tubifex</u>	3.74 5.39 6.80 5.96 <u>9.41</u> $\bar{x}$ 6.26	- - - - -	2.49 4.33 6.91 1.58 <u>1.00</u> $\bar{x}$ 3.26
With <u>P. multisetosus</u>	9.98 6.32 11.33 9.75 <u>10.72</u> $\bar{x}$ 9.62	5.13 10.55 9.97 5.29 <u>14.10</u> $\bar{x}$ 9.01	- - - - -
With both other species	7.95 7.01 13.03 8.09 <u>8.82</u> $\bar{x}$ 8.98	11.06 7.31 10.60 13.45 <u>11.50</u> $\bar{x}$ 10.78	0.49 0.59 1.79 1.82 <u>3.09</u> $\bar{x}$ 1.56

Table 9. Gain in weight of pairs of tubificid species and of a mixture of all 3 after 6 months expressed as multiples of the original weights.

Culture conditions	Mixture of species			
	<u>L. hoffmeisteri</u> plus <u>T. tubifex</u>	<u>L. hoffmeisteri</u> plus <u>P. multisetosus</u>	<u>T. tubifex</u> plus <u>P. multisetosus</u>	All 3 species
Total for 2 or 3 species	3.98 5.34 6.40 6.31 <u>8.53</u> $\bar{x}$ 6.11	8.75 5.57 8.88 8.43 <u>9.30</u> $\bar{x}$ 8.19	4.14 7.61 9.32 4.04 <u>8.03</u> $\bar{x}$ 6.75	7.67 6.54 10.31 8.83 <u>8.44</u> $\bar{x}$ 8.36
Total for 2 species in a mixture of all 3	8.83 7.12 11.73 10.04 <u>10.38</u> $\bar{x}$ 9.62	6.57 6.18 10.07 6.85 <u>5.93</u> $\bar{x}$ 7.12	7.24 5.91 8.44 9.66 <u>8.26</u> $\bar{x}$ 7.90	- - - - -

mixture of all 3 species. All of this indicates considerable influence by P. multisetosus on the other 2 species under a variety of conditions, whereas its own growth is not significantly changed by the presence of either or both of the others.

The total production of worm tissue when two species are combined in a single culture may now be considered (Table 9). No significant differences in growth were observed between the 3 possible pairs, but more worm tissue was produced by any of the pairs than by T. tubifex or P. multisetosus alone ($P < 0.01$). Growth of L. hoffmeisteri alone was not significantly different from that of worms in 2 of the 3 pairs, but L. hoffmeisteri with P. multisetosus produced more worm ($P < 0.05$).

Again when the production of worms in paired culture is compared to the growth of the same 2 in the presence of the third, the presence of P. multisetosus stimulated more growth by the other two than they achieved in its absence ($P = 0.011$).

The mixture of all 3 species produced more worm tissue than any species alone ($P < 0.01$ for T. tubifex, P. multisetosus; $P < 0.05$ for L. hoffmeisteri) and significantly better than the L. hoffmeisteri-T. tubifex culture ($P < 0.05$), but there was no significant difference between the complete mixture and the other paired cultures.

Egestion

The % organic matter in the faeces of T. tubifex and L. hoffmeisteri is significantly higher than that of the original mud at all four experimental temperatures (Table 10). Differences between the two worm species, and temperature effects were of little significance throughout, and the fact that the mud used at 5°C had a lower organic content than that in the other experiments had no effect either. The isolated results obtained using P. multisetosus showed the same trend.

The % nitrogen was significantly higher in the faeces than in the mud at 5°C ($P < 0.01$) and at 10°C and 15°C ($P < 0.05$) for both species (Table 2), but P values at 20°C were 0.05 and 0.86 for L. hoffmeisteri and T. tubifex respectively for the same comparison. There was no significant difference in nitrogen values of the two sets of faeces, and no significant effects due to temperature other than a slightly elevated nitrogen level in the mud at 5°C, probably related to the somewhat higher value in the mud used at that temperature. The five values obtained from faeces of P. multisetosus were about the same as those for mud except for one at 15°C, which was higher.

Table 10. The nitrogen, organic matter and calorific content of feces of T. tubifex and L. hoffmeisteri at 4 temperatures using mud <250 μ particle size.

Nitrogen and organic matter expressed as a %. All values are means $\pm$ S.D. N = 10-15 in each instance.

Parameter	Temperature C	Source of sample		
		mud $\pm$ S.D.	feces of <u>L. hoffmeisteri</u> $\pm$ S.D.	feces of <u>T. tubifex</u> $\pm$ S.D.
% organic matter	5	6.7 $\pm$ 0.8	13.2 $\pm$ 1.8	13.2 $\pm$ 3.4
	10	8.0 $\pm$ 1.9	11.0 $\pm$ 1.9	11.4 $\pm$ 1.2
	15	7.2 $\pm$ 0.6	12.9 $\pm$ 1.5	10.3 $\pm$ 1.8
	20	7.4 $\pm$ 1.1	11.3 $\pm$ 1.7	9.9 $\pm$ 1.6
% nitrogen	5	0.23 $\pm$ 0.01	0.29 $\pm$ 0.04	0.27 $\pm$ 0.02
	10	0.22 $\pm$ 0.01	0.29 $\pm$ 0.10	0.25 $\pm$ 0.04
	15	0.22 $\pm$ 0.01	0.25 $\pm$ 0.03	0.25 $\pm$ 0.04
	20	0.21 $\pm$ 0.01	0.24 $\pm$ 0.04	0.21 $\pm$ 0.06
calories/gm	5	243 $\pm$ 8	289 $\pm$ 31	271 $\pm$ 28
	10	220 $\pm$ 33	319 $\pm$ 50	290 $\pm$ 53
	15	248 $\pm$ 22	285 $\pm$ 36	329 $\pm$ 39
	20	236 $\pm$ 6	284 $\pm$ 47	238 $\pm$ 12

The calorific values of faeces were again significantly higher than those of mud for both species at most temperatures (Table 6). The difference was significant with $P < 0.01$ for L. hoffmeisteri at 10°C and for T. tubifex at 15°C , and P values < 0.05 level were obtained for the former at all other temperatures and for the latter at 10°C . There were no significant differences in calorific values of faeces of the worm species, and no significant differences due to temperature. Again, six of the seven values for P. multisetosus faeces were higher than those of the mud.

In the experiment designed to test the effect of varying the particle size used, the % organic matter was still higher in faeces than in the mud (Table 11). The % nitrogen in faeces of L. hoffmeisteri was significantly higher than in the mud in all three instances ($P < 0.01$ with 230 μ screens but < 0.05 with the other sediments), but in the faeces of T. tubifex there was no longer a significant difference in nitrogen levels once the two finer screens were employed ($P < 0.01$ using 250 μ screens just as in the first experiment at 5°C). At no time did the use of fine screens produce a situation in which % organic matter or % nitrogen was lower in the faeces than in the mud used as a food source, despite the fact that there was a significant increase in both parameters for the mud sieved with 120 μ screen in place of the 250 μ screen. No further significant increase was noted when the 63 μ screen was employed. The % organic matter in the faeces of L. hoffmeisteri showed little significant difference in relation to the type of sediment used ($P < 0.05$ for interactions involving the 120 μ screen, but no significant difference between 250 μ and 63 μ assays) and the same was true for T. tubifex. The faeces of both worm species had substantially the same nitrogen content regardless of the screen size in preparing the mud, and no differences in organic content were detectable in comparisons between the species either. When mixed cultures were used, the percentage organic matter in the faeces was not significantly different from that observed in pure cultures but both were significantly higher than that of the whole mud presented as food (Table 12). Furthermore, there is a significant positive correlation between percentage organic content of the original mud and that of faeces ($P < 0.01$ and $P < 0.05$ for pure and mixed culture respectively), which is linear according to regression analysis for the range 9-14% organic matter in the mud.

Defecation rate

Defecation rate was investigated at a number of temperatures using three species, and at a single temperature (10°C) for a mixture of three species. The rate varied considerably from day to day and experiment to experiment (Figs. 14, 15) (Appleby and Brinkhurst, 1970).

Table 11. The % organic matter and % nitrogen content of three types of sediment and of the feces of two species of tubificid worm fed with them. ($\bar{x} \pm \text{S.D.}$, $N = 12-15$).

	% organic matter			% nitrogen		
	<250	<120	<63	<250	<120	<63
Particle size of food (μ)						
mud used as food	6.7 $\pm$ 0.8	8.1 $\pm$ 0.8	8.7 $\pm$ 0.7	0.23 $\pm$ 0.01	0.26 $\pm$ 0.03	0.27 $\pm$ 0.01
feces of <u>L. hoffmeisteri</u>	13.2 $\pm$ 1.8	14.7 $\pm$ 1.8	13.0 $\pm$ 1.6	0.29 $\pm$ 0.04	0.29 $\pm$ 0.05	0.29 $\pm$ 0.03
feces of <u>T. tubifex</u>	13.2 $\pm$ 3.6	13.3 $\pm$ 2.6	15.3 $\pm$ 2.0	0.27 $\pm$ 0.01	0.27 $\pm$ 0.04	0.27 $\pm$ 0.04

Table 12. % organic matter in faeces of a mixed culture of three worm species compared to the content of faeces of the same worms in pure culture and to the original mud. Experiments at 10 C.

Experiment	Mud	Pure culture	Mixed culture
1	8.9	15.0	14.4
2	9.0	17.2	16.0
3	7.8	15.5	17.6
4	13.4	26.3	22.4
5	13.9	26.0	29.2

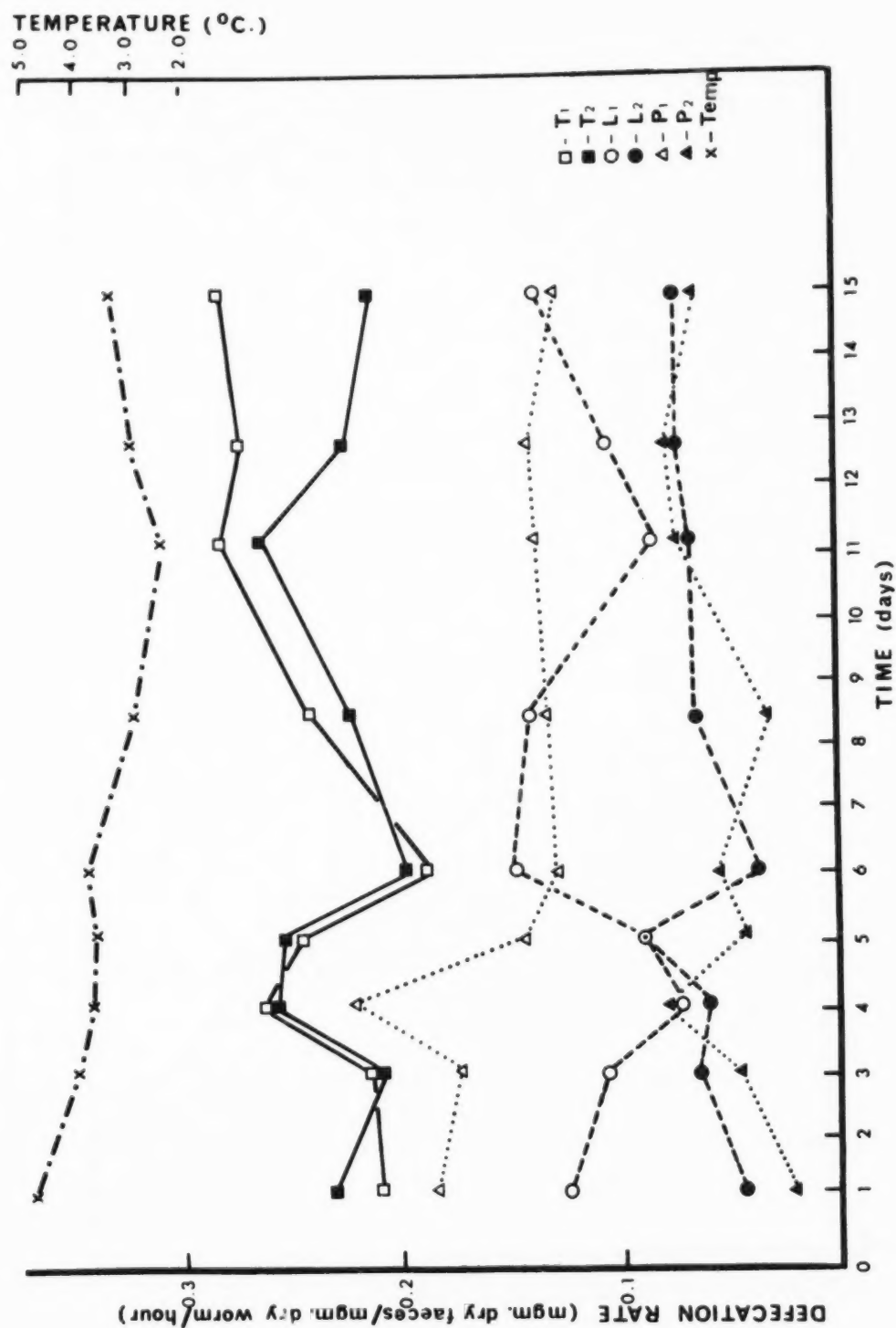


Fig. 14. Defecation rate of worms at a constant temperature of 3.7°C.

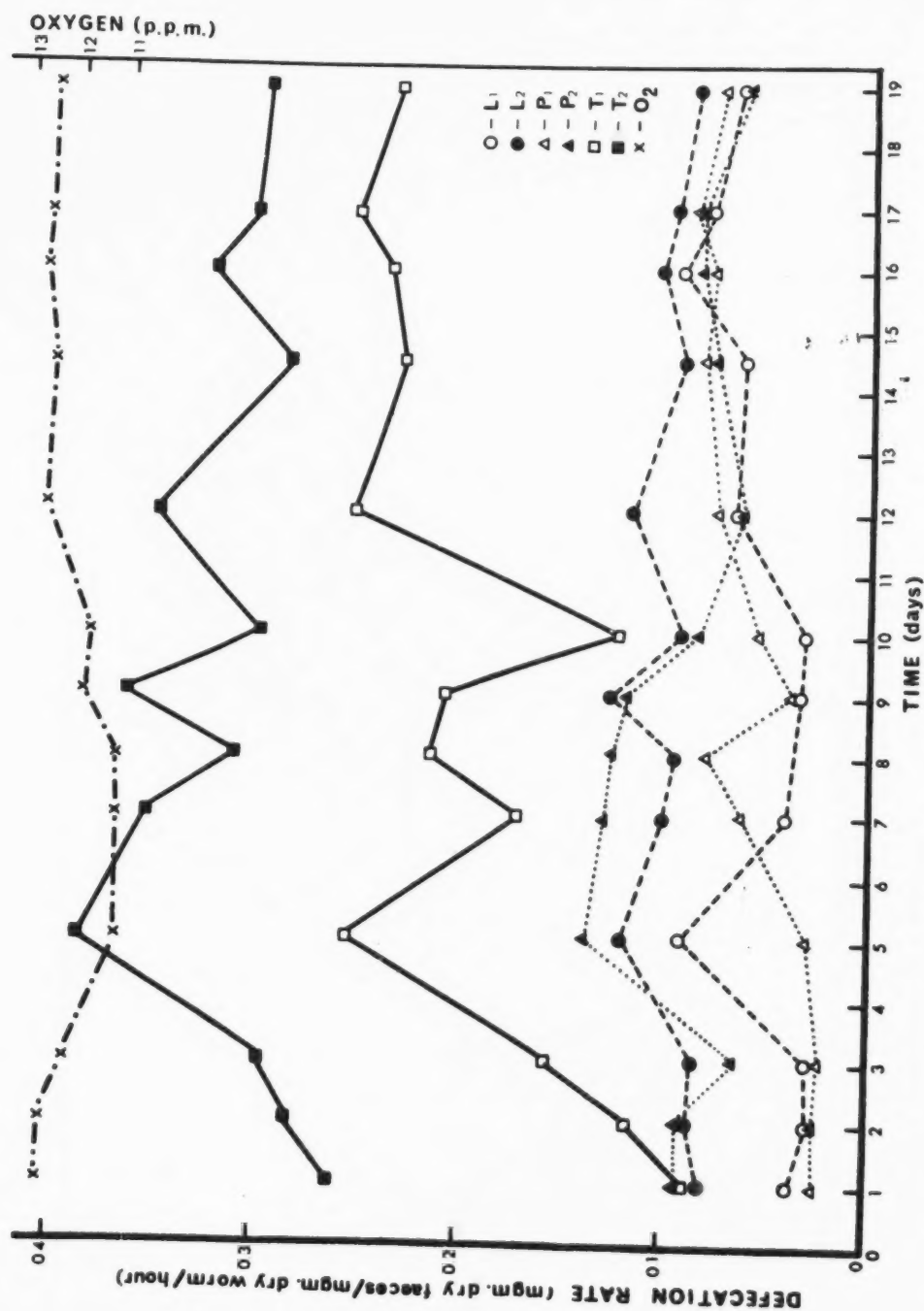


Fig. 15.. Defecation rate of worms at mean temperature of 3.5 C.

In early experiments some relationships between temperature and average defecation rate were established (Fig.16) but these were not duplicated in later studies (those in which the analyses of the faeces were performed) (Table 13). Preliminary efforts to investigate the effect of varying the sediment used as food produced little effect on the defecation rate (Table 14). No data is presented on P. multisetosus in this instance as very few results were obtained, and data presented in Table 13 are based on few positive results for this species.

When the species were mixed together, defecation rate increased by 24% (4.5 mg/mg/day versus 3.6, $n = 35$), a significant difference ($P = 0.03$).

Assimilation

Average calorific values of 5.130, 5.174 and 5.0266 kcal/g were obtained for L. hoffmeisteri, T. tubifex and P. multisetosus respectively. Using this data, the sum of respiration and growth in pure and mixed culture can be calculated.

It should be noted that respiration experiments were of very brief duration, and that growth experiments lasted six months. Also, short-term growth studies suggested that weight losses may occur in 3-week old cultures, so that the true duration of growth experiments might be anything from 20-26 weeks. Nevertheless, on an annual basis the three species in mixed culture might respire 9.38 kcal/g while growth could account for 75.22 kcal/g. In pure culture the three species would respire a total of 13.28 kcal/g but achieve a growth of only 22.5 kcal/g. As there is a greater increase in growth than is accounted for by reduced respiration in mixed culture, the pure culture utilizes less energy than the mixed culture per unit time per unit weight.

Total assimilation of a mixed worm population should be calculated on the basis of 84.6 kcal/gm/year rather than the total of 35.8 kcal/gm/year for three pure cultures. Mean standing stocks of tubificids in Toronto Harbour are probably as high as any that can be recorded anywhere in the world. A survey of eighty-six samples in the harbour yielded the following averages: - 96,000 worms/m², 20.6 g dry wt/m², 18.2 g ash-free dry wt/m². These averages are the result of pooling data from a gradient along which worms varied in abundance from over 125,000 to less than 25,000 (Brinkhurst, 1970). The worms therefore export 200 kcals/m²/annum by virtue of their respiratory activity. The 1550 kcals/m²/annum of worm tissue produced may potentially be returned to the ecosystem via the activities of predators, but some percentage of this quantity is undoubtedly recycled through the worm population when it feeds on bacteria and on the decomposing dead worms. In the Bay of Quinte study the ash-free dry

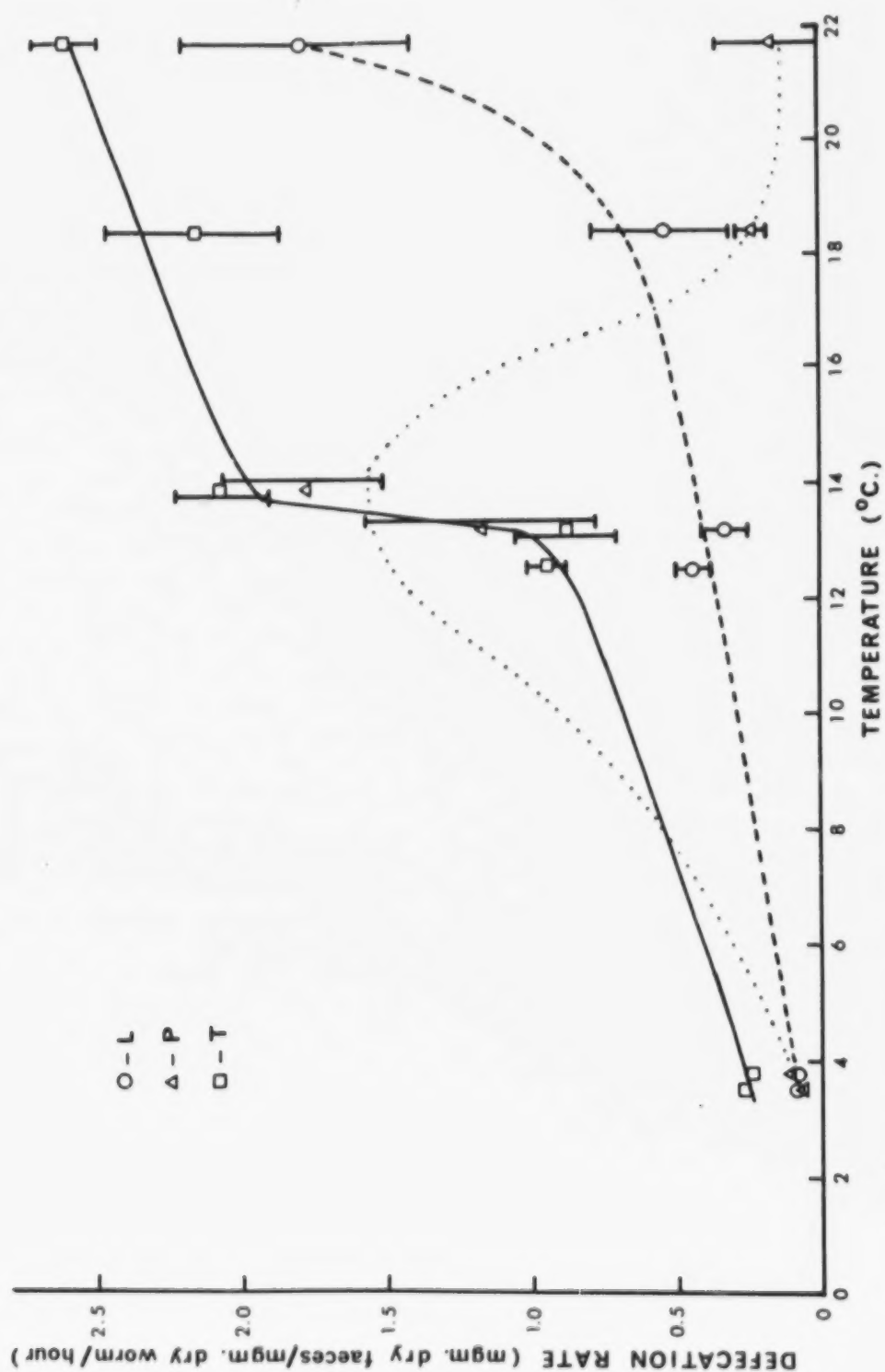


Fig. 16. Possible changes in defecation rate with temperature.

Table 13. Defecation rates for tubificid worms fed on mud with <250 μ particle size ($\bar{x} \pm S.D.$, $n = 10-20$). In mg dry faeces/mg dry worm/day.

Temperature C	Species		
	<u>T. tubifex</u>	<u>L. hoffmeisteri</u>	<u>P. multisetosus*</u>
5	4.2 $\pm$ 2.3	3.4 $\pm$ 1.6	1.2 $\pm$ 1.6
10	3.8 $\pm$ 1.7	5.2 $\pm$ 2.7	3.7 $\pm$ 1.9
15	4.5 $\pm$ 2.2	14.9 $\pm$ 3.7	2.4 $\pm$ 1.3
20	12.7 $\pm$ 10.3	8.1 $\pm$ 5.1	8.1 $\pm$ 4.8

* Data based on few replicates.

Table 14. Defecation rates for tubificid worms fed on three different sediments at 5 C ($\bar{x} \pm \text{S.D.}$). In mg/mg/day.

	Particle size		
	<250 μ	<120 μ	<63 μ
<u>T. tubifex</u>	3.6 $\pm$ 1.7	3.6 $\pm$ 1.2	4.4 $\pm$ 1.5
<u>L. hoffmeisteri</u>	3.4 $\pm$ 1.6	4.0 $\pm$ 2.0	2.8 $\pm$ 1.4

weights per oligochaetes are much lower than for Toronto Harbour (Table 15) where the polluting input probably contains more utilizable input than at Big Bay (see below). The production and assimilation in Toronto Harbour are calculated from respiration data on mixed species complexes, but those for the Bay of Quinte are not. Production data are based on the growth of mixed cultures over six months in the laboratory for Toronto Harbour worms, that for the Bay of Quinte on shorter-term growth determinations also on laboratory held mixed populations. Differences between the various estimates of production and assimilation are roughly proportional to the standing stocks of worms despite the very high levels observed in Toronto Harbour.

Imports

Attempts to study sedimentation rate in Toronto Harbour failed owing to destruction of the equipment by shipping and/or vandals. In a parallel study in the Bay of Quinte samples of various types were evaluated (Johnson and Brinkhurst, 1971c) and all except the narrow (5 cm diameter) traps collected material at roughly the same rate. The small cylinder caught much more than the others ($2.6 \text{ g/m}^2/\text{day}$ as opposed to $0.25\text{-}0.6$).

While correspondence between import and sediment respiration (which includes the living organisms) was good along the trophic gradient from Big Bay to Prince Edward Bay, Lake Ontario, the energy flow into the benthos was not proportional to the total input. At Big Bay much of the $6856 \text{ cal/m}^2/\text{day}$ ends up in the sediment, whereas only 1586 are channelled through the microflora to pass 345 cal on to the benthos. In the less polluted sites lower down the system - imports of 1512-787 cal produce throughputs to the benthos of 1180-420 cal. Hence the quality of the sedimented material is more important than the overall total measured simply in terms of calories, carbon or nitrogen. Selective feeding has already been established by studying the composition of worm faeces. Resistance to bacterial decomposition varies according to the biochemical nature of the sedimenting material or allochthonous material (high for wood fibre, low for milk or sewage for example). Hence a direct relationship between total input in calories and the efficacy of the worm population in recycling this cannot be made. Estimates of worm production and assimilation in a grossly polluted situation can be made (with certain assumption) but these cannot yet be related to total pollutional loading.

The measure of the amount of organic matter in the sediment is equivalent to determination of standing stocks of organisms. Organic material is being added to and removed from the sediment by sedimentation and the activities of the organisms inhabiting it (the dead

Table 15. Standing stock (g ash-free dry weight/m²), annual production and assimilation by oligochaetes (in kcal/m²/yr) at four stations in the Bay of Quinte and in Toronto Harbour. (Values for Bay of Quinte means for 2 annual periods during 1967-68).

	Big Bay	Glenora	Conway	Lake Ontario	Toronto Harbour
Production	5.9	90.0	4.6	2.8	1,549.5
Assimilation	8.6	132.5	9.8	7.0	1,742.8
$\bar{x}$ g ash-free dry wt/m ²	0.12	1.61	0.36	0.32	18.3

bodies and excreta of the same returning materials to the sediment continuously). In our initial work with concentrations and flux rates of amino acids and sugars in the sediment and the overlying water column, it is apparent that flux rates and ambient concentrations are in no way correlated. Hence one cannot judge the activity of an aquatic system by examining the concentration of materials in storage within it. Some knowledge of flows around (for materials) or through (for energy) the system is required in order to compare activity.

In the Bay of Quinte study, macrobenthos biomass appeared to be adjusted to the utilizable import consistent with a turnover rate determined by temperature i.e. $P = \frac{B}{10} T^2$

where P = production, B = biomass, T = mean temperature. Standing stock and production were not closely related in this study, but may be used in relation to temperature data to predict production levels in the Great Lakes (Johnson and Brinkhurst, 1971c).

Nitrogen

We cannot therefore attempt to fulfill our original objective in terms of calories, but as it has been calculated that the contribution of nitrogen to Toronto Harbour might be 830 tons per annum in 1969 (data from Ontario Water Resources Commission, Ontario - Brinkhurst, 1970), we could examine the use of nitrogen by the worm population.

A tubificid worm consists of between 55 to 60 percent protein (Whitten and Goodnight, 1966) and nitrogen is 16 percent of protein. The average standing stock of worms in Toronto Harbour is 20.7 g/m² dry weight, of which 11.8 g/m² (57%) is protein, 1.89 g/m² is nitrogen. The annual turnover rate is about fifteen times the original standing stock for mixed cultures (Table 9) which gives us an annual throughput of nitrogen of 28.35 g/m² in Toronto Harbour. The total area of the harbour is 3,728,000 m², so that the standing stock and throughput of nitrogen overall represents 7046 kg and 105,689 kg respectively, i.e. roughly 7 and 106 tons in relation to the total input of 830 tons. As this imported tonnage enters at one restricted point in the harbour (the Don River - Fig.1) it is not uniformly available to the worm population. Much of it must be sedimented and probably thereby exported in the dredging operation that is carried out in that area. However, the densest worm populations are also in that neighbourhood, with a standing stock of 42.7 gm (250,000 worms) per m² (and hence a minimum nitrogen concentration and throughput of 3.9 g/m² and 58.5 g/m² or about twice the average). The nitrogen utilization of this zone of Toronto Harbour would approximate to 1.5 tons or 22 tons if the area of the zone be taken to be one tenth of the whole harbour.

SECTION VII

DISCUSSION

There are a number of implications to be derived from a consideration of the results obtained herein, and these relate to a set of assumptions inherent in many of the studies of energy flow in ecosystems.

The quality as well as the quantity of material entering a system as allochthonous material or produced within it as autochthonous material is important in determining the nature of the organisms present and the efficiency of cycling material or passaging energy through the system. In the Bay of Quinte maximum production of benthos does not occur where import in terms of carbon or calories is greatest. Bacterial production occurs in nature in a multiple-substrate system in which the biochemical identity of the substrates is more important than the total amount of carbon or energy resources available. Worms, too, exhibit selectivity in their feeding as indicated by the apparent increase in organic matter, nitrogen and calorific value of faeces as opposed to the whole mud upon which they were fed.

Tearing an ecosystem apart and determining energy flow in the laboratory species by species ignores the possibility of interspecific interaction. Our work on mixed cultures makes it clear that respiration and growth of mixed cultures of worms are significantly different from the values obtained when those species, or even those same individuals, are maintained in isolation. How general this sort of effect is we do not know, but we suspect it may involve other benthic organisms at least.

In our study of the use of bacteria as food for worms (Wavre and Brinkhurst, 1970) we indicated that worm species differ in their ability to digest the spectrum of bacteria available to them so that, even if there is no discrimination at ingestion, the faeces differ in bacterial composition. It is possible to conceive of a system in which worms actively seek out the faeces of other species which may contain a high proportion of a preferred bacterium. If that were so, worms would spend time searching rather than eating in the absence of other species.

As the reduction in respiratory energy flow in mixed cultures is less than the weight gain, the suggestion is that more time was spent feeding than burrowing in the mixed culture. The energy saved in respiration added to that obtained by production in pure culture is

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less than the production value in mixed culture, so that there has been an increase in food ingested and/or the efficiency of its utilization. In order to test this hypothesis, we examined defecation rates in mixed and pure populations at the same time. We found that the organic matter in faeces did not change but that there was a 24% greater flow of faeces in mixed populations than in pure. Furthermore, the assimilation efficiency (assimilation in relation to ingestion) increased by over 6%, indicating that a preferred food had been ingested or that some other factor increased its assimilability.

These changes indicate that energy and materials budgets for species cannot simply be summed to yield budgets for species associations found in nature. In order to determine the effect of the sludge-worm population on the harbour ecosystem we would have to know the quantity of useable imported material arriving at the sediments along the gradient Keating Channel - Hanlan's Point, the total being of relatively little significance. We would also have to know the rate of utilization of the worm population by predators and the degree to which worm tissue is simply recycled through the bacteria after death.

The production rates and nitrogen values established allow us to suggest that such large worm populations have a significant potential as sources of material and energy retrieval from what we might be tempted to regard as a sink. The effect of irrigation of those sediments by the worms further enhances their importance in all probability.

SECTION VIII

ACKNOWLEDGEMENTS

The work reported here was carried out under the direction of the Project Director, Drs. K.E. Chua, M.G. Johnson, N. Kaushik, and L.W. Wood. Mrs. C.C. Ho and Mrs. A. Ma provided technical assistance and the secretarial work was undertaken by Mrs. I. Govers and Mrs. M. Thinh. Dr. J. Paloheimo provided statistical advice. Miss S. Dymond Q.C. was of inestimable assistance in preparing applications and reports.

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SECTION X

PUBLICATIONS

Publications produced as a result of this project.

- BRINKHURST, R.O., and K.E. CHUA. (In Prep.). Production studies of tubificid worms in pure and mixed cultures.
- BRINKHURST, R.O., K.E. CHUA, and N.K. KAUSHIK. 1972. Interspecific interactions and selective feeding by tubificid oligochaetes. *Limnol. Oceanogr.* 17: 122-133.
- CHUA, K.E., and R.O. BRINKHURST. (In Prep.). Influence of interspecific interactions on the respiration of tubificid oligochaetes.
- WAVRE, N., and R.O. BRINKHURST. 1971. Interactions between some tubificid oligochaetes and bacteria found in the sediments of Toronto Harbour, Ontario. *J. Fish. Res. Bd Canada* 28: 335-341.

Publications produced as a result of associated projects.

- APPLEBY, A.G., and R.O. BRINKHURST. 1970. Defecation rate of three tubificid oligochaetes found in the sediment of Toronto Harbour, Ontario. *J. Fish. Res. Bd Canada* 27: 1971-1982.
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SECTION XI

APPENDIX

Appendix 1	Temperature and oxygen data
Appendix 2	Percent N ₂ and organic matter in sediment samples.

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APPENDIX 1

Temperature and Oxygen Concentrations of top and bottom water at sampling site (March - December, 1969 - measurement taken between 1000 to 1400 hours). Data from Toronto Harbour (Hanlan's Point).

Date	Temperature °C		Oxygen Conc. (% saturation)	
	Top	Bottom	Top	Bottom
19/3/69	1.5	2.5	ca.130	ca.130
14/4/69	9.8	6.2	112	97
30/4/69	10.0	8.6	115	98
15/5/69	12.2	8.9	125	104
27/5/69	13.4	10.8	127	111
4/6/69	11.4	10.0	98	92
11/6/69	14.4	11.8	122	108
18/6/69	14.2	12.6	105	95
25/6/69	15.6	12.7	109	85
2/7/69	16.5	15.0	119	76
9/7/69	17.0	14.9	132	90
16/7/69	20.8	17.5	112	65
23/7/69	21.8	20.4	117	72
30/7/69	21.7	20.8	65	25
7/8/69	23.4	20.4	90	9
12/8/69	20.0	17.0	117	49
18/8/69	18.6	16.5	130	80
27/8/69	19.8	16.6	116	53
4/9/69	21.0	19.4	117	87
10/9/69	19.7	17.3	99	51
19/9/69	13.8	11.8	133	100
29/9/69	15.4	14.4	116	94
22/10/69	8.2	8.0	105	99
13/10/69	7.1	7.1	107	105
24/11/69	4.0	4.0	107	107
22/12/69	0.60	0.70	125	125

APPENDIX 2. A.

Percentage of N_2 by weight of dry sediment (60 C) ($\bar{x} \pm 95\%$ c.l., $n = 6$) Samples from

Toronto Harbour Zones 1-4, July, August, September 1970.

Zone 1.	July 17, 1970	August 18, 1970	September 18, 1970
0-2 cm	0.161 $\pm$ 0.005	0.177 $\pm$ 0.027	0.171 $\pm$ 0.007
2-4 cm	0.140 $\pm$ 0.004	0.176 $\pm$ 0.027	0.153 $\pm$ 0.021
4-6 cm	0.140 $\pm$ 0.005	0.166 $\pm$ 0.028	0.165 $\pm$ 0.029
6-8 cm	0.140 $\pm$ 0.004	0.159 $\pm$ 0.030	0.167 $\pm$ 0.014
8-10 cm	0.124 $\pm$ 0.004	0.157 $\pm$ 0.040	0.170 $\pm$ 0.010
Zone 2.			
0-2 cm	0.135 $\pm$ 0.009	0.160 $\pm$ 0.008	0.177 $\pm$ 0.015
2-4 cm	0.119 $\pm$ 0.007	0.158 $\pm$ 0.006	0.155 $\pm$ 0.004
4-6 cm	0.109 $\pm$ 0.005	0.156 $\pm$ 0.010	0.142 $\pm$ 0.011
6-8 cm	0.120 $\pm$ 0.008	0.148 $\pm$ 0.017	0.134 $\pm$ 0.009
8-10	0.102 $\pm$ 0.006	0.138 $\pm$ 0.012	0.129 $\pm$ 0.004
Zone 3.			
0-2 cm	0.212 $\pm$ 0.030	0.202 $\pm$ 0.013	0.162 $\pm$ 0.019
2-4 cm	0.205 $\pm$ 0.010	0.185 $\pm$ 0.008	0.157 $\pm$ 0.021
4-6 cm	0.183 $\pm$ 0.010	0.188 $\pm$ 0.005	0.157 $\pm$ 0.015
6-8 cm	0.176 $\pm$ 0.015	0.175 $\pm$ 0.004	0.159 $\pm$ 0.010
8-10 cm	0.169 $\pm$ 0.006	0.166 $\pm$ 0.009	0.173 $\pm$ 0.008
Zone 4.			
0-2 cm	0.345 $\pm$ 0.034	0.343 $\pm$ 0.013	0.383 $\pm$ 0.027
2-4 cm	0.324 $\pm$ 0.021	0.321 $\pm$ 0.019	0.357 $\pm$ 0.026
4-6 cm	0.275 $\pm$ 0.008	0.275 $\pm$ 0.036	0.316 $\pm$ 0.018
6-8 cm	0.173 $\pm$ 0.017	0.204 $\pm$ 0.014	0.278 $\pm$ 0.011
8-10 cm	0.129 $\pm$ 0.022	0.197 $\pm$ 0.010	0.219 $\pm$ 0.026

APPENDIX 2. B.

Percentage of organic matter in samples of sediment from Toronto Harbour Mean values + 95% confidence limits. Sediment dried at 60 C ignited at 500 C. No corrections applied. Zones 1-4, July, August, September 1970.

Zone 1.	July 17, 1970	August 18, 1970	September 18, 1970
0-2 cm	6.90 $\pm$ 0.16	9.15 $\pm$ 0.95	7.30 $\pm$ 0.27
2-4 cm	6.50 $\pm$ 0.27	9.24 $\pm$ 0.37	7.38 $\pm$ 0.41
4-6 cm	6.98 $\pm$ 0.69	8.54 $\pm$ 0.52	6.69 $\pm$ 1.64
6-8 cm	7.21 $\pm$ 0.65	7.40 $\pm$ 0.49	7.32 $\pm$ 0.82
8-10 cm	6.28 $\pm$ 0.33	8.24 $\pm$ 0.64	7.26 $\pm$ 0.25
Zone 2.			
0-2 cm	6.15 $\pm$ 0.49	6.99 $\pm$ 0.26	7.07 $\pm$ 0.37
2-4 cm	5.79 $\pm$ 0.27	6.68 $\pm$ 0.15	6.49 $\pm$ 0.21
4-6 cm	5.68 $\pm$ 0.60	6.50 $\pm$ 0.19	6.46 $\pm$ 0.34
6-8 cm	6.20 $\pm$ 0.24	6.00 $\pm$ 0.45	6.30 $\pm$ 0.29
8-10 cm	5.24 $\pm$ 0.27	5.86 $\pm$ 0.47	6.18 $\pm$ 0.28
Zone 3.			
0-2 cm	7.45 $\pm$ 0.70	7.63 $\pm$ 0.44	7.80 $\pm$ 0.33
2-4 cm	7.25 $\pm$ 0.58	7.08 $\pm$ 0.16	7.91 $\pm$ 0.32
4-6 cm	7.65 $\pm$ 0.24	7.13 $\pm$ 0.07	8.12 $\pm$ 0.22
6-8 cm	7.61 $\pm$ 0.28	6.89 $\pm$ 0.28	8.18 $\pm$ 0.36
8-10 cm	7.13 $\pm$ 0.21	6.62 $\pm$ 0.20	7.98 $\pm$ 0.35
Zone 4.			
0-2 cm	10.32 $\pm$ 0.79	9.54 $\pm$ 0.19	9.84 $\pm$ 0.27
2-4 cm	9.52 $\pm$ 0.34	8.60 $\pm$ 0.40	9.55 $\pm$ 0.34
4-6 cm	8.33 $\pm$ 0.43	7.75 $\pm$ 0.93	8.80 $\pm$ 0.31
6-8 cm	5.44 $\pm$ 0.71	6.28 $\pm$ 0.42	8.28 $\pm$ 0.38
8-10 cm	4.09 $\pm$ 0.72	6.49 $\pm$ 0.42	6.63 $\pm$ 1.00